In Table IIC, it will be noticed that the excretion of Lot 80 at 6 hours was slower than that of the other light gelatin lots, and that correspondingly the excretion of Lot 80 after 6 hours up to 72 hours was greater than that of the other lots. All 4 of these subjects treated with this lot of gelatin showed a moderate pyrogenic reaction at the end of the excretion. The reactions were not severe, and they occurred so near the end of the injection, that the experiments were carried through to completion. The excretions of gelatin in these cases for the period up to the end of the injection were of the same order as that for the other light lots. But for the next 6 hours there was a markedly diminished gelatin output. That this decrease was due to diminished renal function was indicated by the fact that the creatinine clearances (Table IIIC) were appreciably lower than normal. In three of the four cases, this diminished excretion was compensated for by an unusually large excretion during the remainder of the first day. In the fourth case, the excretion was delayed to the third day (and fourth day, which is not shown in the Table). If Lot 80 is omitted entirely from the calculation of the excretion of light gelatin at 6 hours, the average excretion at 6 hours is increased from 22.72 to 24.66 grams, and the difference between this excretion and the average for heavy gelatin at 6 hours becomes even greater.

Gelatin and creatinine clearances. Tables IIIA, IIIB, and IIIC show the endogenous creatinine and gelatin clearances and gelatin-creatinine ratios for 12 heavy, 9 intermediate, and 11 light gelatin experiments. Though all subjects chosen were thought to have normal renal function, a few showed abnormally low and an occasional one abnormally high creatinine clearances. These abnormalities could for the most part be accounted for by the fact that catheterization was not resorted to, and that volume errors occurred, in one direction in the first two hours and in the reverse direction in the second. Some of the abnormality might have been due to the effect of gelatin injection. On the one hand, the increased plasma volume produced by the gelatin injection should have produced a greater renal flow and a greater glomerular filtration. On the other hand, the excretion of a highly concentrated and viscous gelatin-containing urine might temporarily block a number of tubules and thus temporarily reduce renal function. It is significant that the large ma-

TABLE IIIA

Gelatin and creatinine clearances for injection of "heavy" gelatin

Lot no.	Patient	0 to 2 hrs. after injection			2 to 4 hrs. after injection			4 to 6 hrs. after injection		
		Gelatin clearance G	Creatinine clearance C	G/C (×100)	Gelatin clearance G	Creatinine clearance C	G/C (×100)	Gelatin clearance G	Creatinine clearance C	G/C (×100)
		ml. per min.	*ml. per min.*		*ml. per min.*	*ml. per min.*		*ml. per min.*	*ml. per min.*	
58–10	A.H.	4.33	86.4	5.01	8.26	170	4.85	3.59	86.5	4.15
	J.D.	1.67	43.2	3.87	2.46	83.6	2.94	5.08	133.8	3.80
	A.W.	7.68	136	5.65	3.85	135	2.85	1.50	97.8	1.53
	H.M.	4.03	146	2.74	6.93	135	5.14	4.45	151	2.94
	C.T.	6.63	171	3.88	3.17	177	1.79	6.40	162	3.95
	F.B.	6.03	93.5	6.45	5.09	112.5	4.52	4.59	207	2.21
	O.B.	2.09	61.5	3.40	2.66	112	2.37	1.95	111	1.76
93	C.W.	4.78	134	3.57	3.25	112	2.90	1.45	138	1.05
	R.R.	6.96	182	3.42	2.07	137	1.51			
	J.R.	2.59	133	1.95	2.94	126	2.33			
	D.C.	6.58	122	5.40	3.11	118	2.64	1.89	93.3	2.03
	P.W.	0.636*	36*	1.77*	0.636*	36*	1.77*	1.63	123	1.33
83	W.H.	3.39	86.5	3.92	3.72	134	2.78	5.42	92.3	5.87
Average and standard deviation				3.93 ±1.45			2.95 ±1.13			2.78 ±1.25

t (Mean G/C for 0 to 2 hours compared with mean G/C for 2 to 4 hours) 1.9.
t (Mean G/C for 2 to 4 hours compared with mean G/C for 4 to 6 hours) 0.4.
* Single clearance test over a period of 4 hours.

TABLE IIIB

Gelatin and creatinine clearances for injection of "intermediate" gelatin

Lot no.	Patient	0 to 2 hrs. after injection			2 to 4 hrs. after injection			4 to 6 hrs. after injection		
		Gelatin clearance G	Creatinine clearance C	G/C (×100)	Gelatin clearance G	Creatinine clearance C	G/C (×100)	Gelatin clearance G	Creatinine clearance C	G/C (×100)
		ml. per min.	*ml. per min.*		*ml. per min.*	*ml. per min.*		*ml. per min.*	*ml. per min.*	
58–15	U.B.	2.78	126	2.21	3.16	113	2.79	2.88	116	2.48
	S.S.	5.59	56.2	8.09	6.83	65.4	10.44	2.18	55.5	3.93
	H.C.	7.34	118	6.23	3.40	159	2.14	1.62	119	1.36
	F.F.	5.23	269	1.94	8.31	227	3.66	4.04	99	4.08
101	J.M.	8.18	117	7.02	4.52*	124*	3.65*	4.52*	124*	3.65*
	W.C.	14.4	125	11.50	6.74	111	6.09	1.64	88.5	1.85
	F.B.	14.8	151.5	9.78	6.32	180	3.51	4.18	196	2.13
	H.M.	4.88*	114*	4.28*	4.88*	114*	4.28*	5.31	130	4.09
	R.Y.	12.48	151	8.24	4.64	105	4.41	3.86	111	3.48
Average and standard deviation				6.59 ±3.06			4.55 ±2.32			3.01 ± .99

t (Mean G/C for 0 to 2 hours compared with mean G/C for 2 to 4 hours) 1.6.
t (Mean G/C for 2 to 4 hours compared with mean G/C for 4 to 6 hours) 1.8.
* Single clearance test over a period of 4 hours.

TABLE IIIC

Gelatin and creatinine clearances for injections of "light" gelatin

Lot no.	Patient	0 to 2 hrs. after injection			2 to 4 hrs. after injection			4 to 6 hrs. after injection		
		Gelatin clearance G	Creatinine clearance C	G/C (×100)	Gelatin clearance G	Creatinine clearance C	G/C (×100)	Gelatin clearance G	Creatinine clearance C	G/C (×100)
		ml. per min.	*ml. per min.*		*ml. per min.*	*ml. per min.*		*ml. per min.*	*ml. per min.*	
65	L.M.	4.85	57.1	8.50	3.86	63.3	6.10	6.75	144	4.69
	L.P.	12.72	91.7	13.88	7.02	135.5	5.18	2.84	97.9	2.90
	Lu.M.	12.77	91.3	13.98	10.12	105.1	9.63	3.17	68.3	4.64
39	C.U.	14.4	161	8.95	6.22	110	5.66	3.11	89	3.50
	L.B.	13.3	147	9.05	5.02	131	3.83	2.97	115	2.58
	J.C.	9.18	134	6.85	5.75	192	3.00	8.23	154	5.34
45	C.P.	11.78	102	11.52	4.16	126	3.30	1.80	116	1.55
	S.M.	7.23	89.7	8.06	6.23	92.5	6.73	2.67	70.1	3.81
80	H.R.	3.50	33.0	10.61	3.89	39.8	9.77	1.75	45.3	3.86
	J.S.	6.00	67.2	8.93	4.82	86.4	5.58	5.06	65.3	4.75
	A.D.	7.47	96.5	7.74	3.01	93.5	3.22	5.51	97.5	5.65
Average and standard deviation				9.82 ±2.29			5.64 ±2.26			3.93 ±1.18

t (Mean G/C for 0 to 2 hours compared with mean G/C for 2 to 4 hours) 4.1.
t (Mean G/C for 2 to 4 hours compared with mean G/C for 4 to 6 hours) 2.9.

jority of subjects showed creatinine clearances within 130 ± 25 ml. per minute.

If a comparison is to be made of the gelatin clearances in the various intervals in the same individual, or of those in same interval in the several subjects, it is necessary to nullify the effect of variations in renal functions. This nulli-fication can be accomplished by making use of ratio of the gelatin clearance to the creatinine clearance. This ratio multiplied by 100 is the gelatin clearance in percentage of the creatinine clearance. This value (expressed as G/C in the charts) should be a measure of the relative ease of glomerular filtration of gelatin in any particular ex-

periment as compared with a crystalloid, the clearance of which is supposed to be a measure of glomerular filtration. Though it is known that in man creatinine clearance is larger and more variable than the clearance of a substance like inulin which is not resorbed or excreted by the tubules, in an experiment such as this, where inulin clearances are too inconvenient to perform, creatinine clearances are probably satisfactory as measures of glomerular filtration.

There was a marked difference between the G/C values for heavy gelatin and those for light gelatin as seen in Tables IIIA, IIIB, and IIIC. For the former, for the 0 to 2 hour period, G/C ranged from 1.77 to 6.45 with an average of 3.93, and a standard deviation of ± 1.45. For the 2 to 4 hour period, the average G/C value was 2.95, with a standard deviation of ± 1.13; and for the 4 to 6 hour period the average was 2.78 and the standard deviation ± 1.25. The t value when the mean G/C for 0 to 2 hours was compared with the mean G/C for 2 to 4 hours was 1.9; but for the comparison of the 2 to 4 hour and the 4 to 6-hour periods, the t value was 0.4. Thus, for the heavy gelatin, though there appeared to be a progressive reduction in G/C value during the 6 hour period following the end of the injection, the change was not significantly great.

On the other hand, in the case of the light gelatin, G/C for the 0 to 2-hour period was much higher. It ranged from 6.85 to 13.98, and averaged 9.82, with a standard deviation of ± 2.29. There was a significant drop in G/C values in the second period to an average of 5.64, and still further drop in the third 2-hour period to an average of 3.93. That these declines in G/C values were genuine was seen from the t values of 4.1 and 2.9. Thus, the light gelatin was much more readily excreted through the glomeruli than the
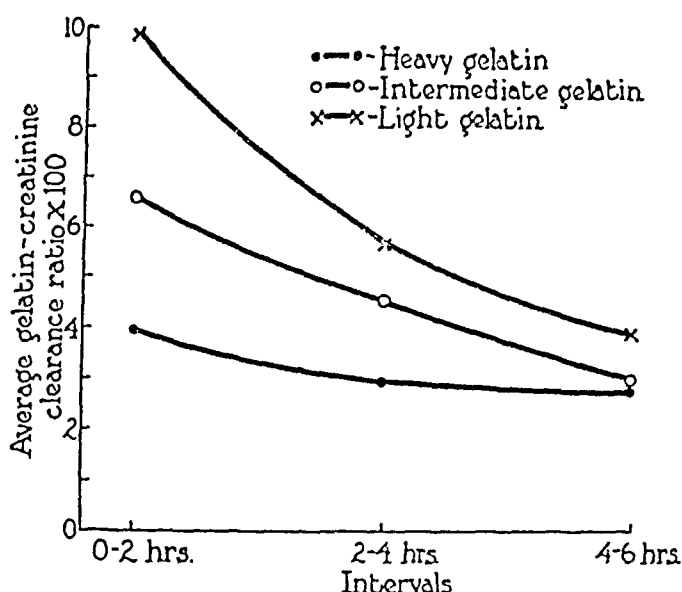

FIG. 2. COMPARISON OF AVERAGE GELATIN-CREATININE CLEARANCE RATIOS IN THREE SUCCESSIVE 2-HOUR PERIODS AFTER THE INJECTION OF HEAVY, INTERMEDIATE, AND LIGHT GELATINS RESPECTIVELY

heavy gelatin during the first 2 hours, but by the end of 6 hours, its relative clearance approached that of heavy gelatin (See Figure 2).

The intermediate gelatin, as indicated in the other data, showed a much greater variability in excretion, but the values appear to lie in between those of light gelatin and those of heavy gelatin. The mean G/C values for the three periods and their respective standard deviations were 6.59 ± 3.06, 4.55 ± 2.32, and 3.01 ± 0.99. Here too, the progressive drop in G/C until the values were in the range of those of heavy gelatin was apparent.

Plasma volume and the distribution of gelatin. Plasma volume determinations were made in only a few cases toward the end of the investigation. Determinations were made before the beginning of the experiment and at the end of the injection. The data in 8 cases, shown in Table V, are too

TABLE IV

Water excretion and urinary gelatin concentrations for 6 hours following the intravenous injection of 5 per cent gelatin solution

Type of gelatin	Light					Heavy		Intermediate	
Lot nos.	39	65	45	80	83	93	58–10	58–15	101
Average water excretion to 6 hours after injection, ml.	1538	889	621	779	445	955	835	846	1239
Maximal water excretion, ml.	1780	1230	925	1402	445	1577	1493	1542	1725
Minimal water excretion, ml.	1345	510	317	272	445	600	422	325	325
Maximal gelatin concentration, grams per 100 ml.	3.42	10.26	12.06	5.77	4.71	6.18	4.16	7.96	14.96

TABLE V

Distribution of gelatin at the end of injection of 50 grams of gelatin

Subject	Weight	Gelatin type and lot	Initial plasma volume	Plasma volume at end of injection	Plasma gelatin concentration	Total plasma gelatin	Total urinary gelatin	Total tissue gelatin
	kgm.		*ml.*	*ml.*	*grams per ml.*	*grams*	*grams*	*grams*
D.C.	71	Heavy 93	3333	3846	0.929	35.72	6.20	8.08
C.W.	50	Heavy 93	2000	3030	0.792	24.00	17.45	8.55
P.W.	53	Heavy 93	2424	3571	0.684	24.43	8.88	17.69
C.P.	55	Light 45	2410	3125	0.838	26.19	8.84	14.97
J.R.	45	Heavy 93	1887	3704	0.741	27.44	6.89	15.67
R.R.	74	Heavy 93	3125	4273	0.770	32.90	14.63	2.47
J.C.	67	Light 39	2778	3680	0.593	21.82	18.24	9.94
L.B.	50	Light 39	2062	3555	0.780	27.73	12.19	10.08
Average						27.53	11.66	10.87

meager for statistical analysis, but they at least show a trend. They demonstrate the well established hemodiluting effect of injected gelatin. From the plasma volume and the plasma gelatin concentration at the end of the injection, the total circulating gelatin could be calculated. Since the urinary gelatin during the period of injection was known, the difference between the sum of these factors and the total injected (taken as 50 grams in each case [5]) gave the amount of gelatin unaccounted for, which probably was in the interstitial fluids, since it was continuously excreted apparently unchanged during the following days. If the 8 cases were representative of all cases, then only some 55 per cent of the gelatin was still in the blood stream at the end of the injection, some 23 per cent had already been excreted, and some 22 per cent had been filtered off into the tissues. These findings are in close agreement with the findings of Little and Dameron (13), who demonstrated that, after a single injection of a Knox preparation of gelatin (labeled Lot number B 78–1's) into normal dogs, only 50 per cent of gelatin was still present in the blood at the end of the injection and that 23 to 35 per cent had been excreted in the urine.

[5] Analysis of the gelatin preparations showed the presence in all specimens of small but appreciable amounts of non-protein nitrogen which when subtracted from the total nitrogen often reduced the concentrations to slightly below 5 per cent. In addition to this error there was always an error due to loss of some solution in the preparation of the intravenous infusion. However both of these errors were at least partly annulled by the fact that the bottles contained slightly more than 1000 ml.

DISCUSSION

Analysis of the data for the plasma gelatin concentration, urinary gelatin excretions and gelatin-creatinine clearance ratios for the three types of gelatin makes discernible a more or less consistent pattern in spite of great variability in the individual experiments. The higher G/C values for the light gelatin in the first hours must be interpreted as being due to a larger proportion of more easily filterable molecules. These are about 10 per cent as readily filterable as creatinine through the glomeruli (and probably also through the general capillary endothelium). In the second and third 2-hour periods, there being a much smaller porportion of easily filterable molecules, the G/C values drop considerably and approach those for the heavy gelatin experiments. In the later hours the clearances may be of the same order for all types, but the slightly higher plasma concentrations, plus possibly a slightly higher plasma volume, permit the compensatorily increased excretion of the heavy gelatin, so that eventually the total excretions are comparable.

The differences between the plasma gelatin concentrations fit in with this interpretation. With smaller excretions in the early period, and probably with similarly smaller transudations into the interstitial fluids, a relatively high plasma concentration is maintained for the heavy gelatin a longer time than for the light gelatin. Why this phenomenon is not demonstrable at the end of the injection is difficult to determine, but it probably has something to do with the relative amounts of hemodilution that occurred with the two types of gelatin. Unfortunately, an insufficient number of

accurate plasma volume determinations were made in these experiments to permit a comparison between the heavy and light types.

An alternative interpretation of the progressive decline of the gelatin-creatinine clearance ratios presents itself, if one can make the unlikely assumption that gelatin is appreciably resorbed by the renal tubules. Pitts and Alexander (14), studying the renal resorptive mechanism of phosphate, showed that the phosphate-creatinine clearance ratios diminished as the plasma phosphate decreased, because the portion of filtered phosphate that was resorbed increased as less phosphate was filtered. To interpret the gelatin excretions on the same basis, one would have to postulate not only that gelatin is freely resorbed by tubules, but that resorption is the same for all sized molecules of gelatin. Neither of these postulates is likely. It is much more reasonable to assume that of molecules ranging in molecular weight from 20,000 to 80,000, the smaller ones will be filtered more rapidly through the glomerular membranes, even if the molecules are elongated elipsoids with all nearly the same narrow diameter. This concept is further strengthened by the fact that when the ultracentrifuge pattern of the gelatin is narrow there is relatively little change in the successive G/C values, and that the values for the three types of gelatin approach each other.

The magnitude of the gelatin clearance is surprising. Even in the case of heavy gelatin, absolute clearances ranging from 1.7 to 7.7 are much larger than might be anticipated from the knowledge of the behavior of a substance like serum albumin, the clearance of which in intact kidneys approaches 0. The clearance of serum albumin even in nephrotic edema was found by Luetscher (15) to be only about 0.85. The explanation for the difference in excretability of the two proteins is of course not a simple one, but one of the important factors must be the globoid shape of the albumin molecule, as contrasted with the elongated elipsoid character of the gelatin molecule.

That the gelatin concentration in the urine may reach 15 grams per 100 ml. indicates (See Table IV), as might be expected, that tubular resorption of water is not significantly deterred by the osmotic effect of gelatin, which effect is small compared with that of the crystalloids of the urine. Simi-

larly high protein concentrations are not infrequently seen in severe cases of nephrotic syndrome associated with oliguria.

From a clinical point of view, the data point to at least a theoretical superiority of the heavy gelatin over the light gelatin. The plasma gelatin concentrations are maintained better in the case of heavy gelatin during the first few hours after the injection, which is the period of the desired clinical effect, whereas the ultimate excretion is as complete as in the case of the light gelatin. Furthermore the heavy gelatin injections are less likely to produce excessively high concentrations of gelatin in the urine, which might temporarily embarrass renal function. On the other hand, there is unequivocal evidence from this laboratory that the injection of light gelatin is clinically effective in shock and is innocuous. From a practical point of view there may be no appreciable difference between the two types. Surely one cannot extend the results of this study to attempt to prove that still heavier gelatin, the type that gels at room temperature, is for clinical purposes even better than the heavy gelatin utilized here, for many factors other than those discussed here, such as inconvenience of administration and danger of deposition in the tissues, must be taken into consideration.

SUMMARY AND CONCLUSIONS

1. The fate of intravenously injected gelatin was studied in 42 hospital control subjects to whom were administered 1000 ml. of 5 per cent gelatin. Though the solutions were all liquid at room temperatures, they were of three types: heavy, intermediate, and light. The weight average molecular weights were respectively of the order of 58,000, 47,000, and 37,000.

2. The plasma gelatin concentration was highest at the end of the injection. It averaged 0.78 gram per 100 ml., and was the same for all three types. At 24 hours the plasma gelatin concentration had dropped to about 0.3 gram per 100 ml. for all three types. For the first six hours after the injection the plasma levels were maintained significantly higher in the heavy gelatin experiments. At 48 and 72 hours there were still appreciable quantities of gelatin in the plasma.

3. The urinary excretion of gelatin was markedly variable even with the same lot, but a pattern

of excretion was discernible. The heavy gelatin excretion in 6 hours after the end , of the injection averaged 14.68 grams, while the light was 22.72 grams. The intermediate gelatin values lay between these. By 72 hours, some 80 per cent of all types of gelatin was excreted. There was still further excretion on the fourth and fifth days. Thus it was unlikely that any considerable quantity of gelatin had been catabolized.

4. The average gelatin-creatinine clearance ratios were much higher for light gelatin than for heavy or intermediate gelatin. During the 3 successive 2-hour periods following the end of the injection, the ratios fell, but much more steeply for the light gelatin, so that at the end of 6 hours the ratios were all of the same order. This phenomenon was interpreted as indicating that the smaller gelatin molecules were rapidly excreted, leaving in the body molecules of the same order of excretability for all three types of gelatin.

5. No diuresis was seen after the injection of gelatin, except possibly where the gelatin was dissolved in 5 per cent dextrose. Concentrations of gelatin in the urine as high as 15 grams per 100 ml. were occasionally encountered which may produce a viscosity great enough to interfere temporarily with renal function. The lowest maximal urinary gelatin concentrations occurred in the heavy gelatin experiments.

6. Plasma volume determinations showed the well established hemodiluting effect of gelatin, and demonstrated that at the end of the injection only about 55 per cent of the gelatin was still in the blood stream, about 23 per cent having been excreted and 22 per cent filtered into the tissues.

7. These results indicate a theoretical clinical superiority of the heavy gelatin over the lighter types. Nevertheless light gelatin has been found to be clinically effective and innocuous in the treatment of shock.

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CHANGES IN BLOOD COAGULATION FOLLOWING CORONARY THROMBOSIS MEASURED BY THE HEPARIN RETARDED CLOTTING TEST (WAUGH AND RUDDICK TEST) [1,2]

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Recently many authors have studied the phenomenon of accelerated coagulability of the blood in a variety of conditions. It is now well known that numerous factors may play a rôle in increasing the coagulability of the blood. This tendency toward an increasing coagulability of the blood will be referred to as "acceleration" throughout this paper. Among factors which may accelerate the coagulation of blood should be mentioned stasis of blood, trauma to vessel walls, dehydration, anemia, and the release of excessive quantities of thromboplastin in the blood such as occur after trauma, surgery, infection, or childbirth. Each of these factors may act independently or in conjunction with the others. De Takats (1) demonstrated a change in the clotting mechanism in a variety of thrombotic states such as venous thrombosis, cerebral thrombosis, arterial embolus and coronary thrombosis. Brambel *et al.* (2) demonstrated a tendency toward acceleration of coagulation using Quick's prothrombin test with 12.5 per cent diluted plasma in crush injuries associated with gangrene, diabetic and arteriosclerotic gangrene and frost bite. Shapiro (3, 4) has also demonstrated a tendency to accelerated coagulability of the blood using diluted plasma, and thought it significant in thromboembolization. He found in 5 cases of embolization a lowered prothrombin time. Hirschboeck (5) found in 9 out of 10 cases of pulmonary embolism a decrease in clot retraction time. Shafiroff *et al.* (6) found, using the Lee-White technique, that blood from a limb affected with thrombosis or phlebitis, as compared with blood taken from a normal limb in the same subject, showed accelerated coagulation.

Doles (7), however, found in 13 cases of coronary thrombosis that 12 to 48 hours after the episode, the prothrombin times in percentage of normal were reduced. Hines and Kessler (8) found in 8 patients with evidence of either coronary or cerebral thrombosis, a tendency to increased coagulation as measured by Quick's prothrombin determination and the De Takats heparin tolerance method (1). They found this in association with a high erythrocyte count. Nay and Barnes (9) have recently reviewed a series of 100 cases of coronary thrombosis, in which complications of a thrombotic or embolic nature occurred in 37 cases.

Heparin has been suggested as a therapeutic measure by Best (10). Experimentally, Solandt and Best (11) prevented coronary thrombosis in previously heparinized dogs. Knowing that anticoagulants are being used in the management of coronary thrombosis as well as other diseases, it seems desirable to know, if possible, whether such patients do indeed have accelerated clotting and to what degree. Furthermore, if such an acceleration were demonstrated, it would be desirable to know the duration of such a change.

Our present study was motivated by an autopsy study by one of us (12) of 100 hospital cases of coronary thrombosis. Extension of the coronary clot in a proximal direction was found in 12 per cent of the cases. In 18 per cent a new thrombosis occurred, probably while the patient was in the hospital. Mural thrombosis on the wall of the ventricle occurred in 60 per cent of the cases, often associated with auricular mural thrombosis. In an additional 5 per cent, atrial mural thrombosis occurred without ventricular mural thrombosis. This gives a total of 65 per cent for all mural thrombosis. Peripheral thrombo-embolic manifestations occurred in 45 per cent of the cases, which came to autopsy because of the consequences of coronary thrombosis. With this in mind, serial

study of blood coagulation was made on coronary thrombosis cases from hospital entry until discharge (or death) to see if some significant alteration did occur.

METHODS OF OBSERVATIONS

Sixteen hospital patients were selected as controls, and 27 different cases of coronary thrombosis were followed serially in our study. The Waugh-Ruddick test (13, 14) was used throughout in order to determine if any alteration of blood coagulation occurred in coronary thrombosis. The studies were made at room temperatures ranging from 70 to 75° F., average 72° (15). Lee-White and capillary clotting times were run simultaneously with the Waugh-Ruddick test. We at first familiarized ourselves with the method on 7 recent convalescents, who had completely recovered from their illness, and were ambulatory most of the day. Our results were in essential agreement with the authors. Frequent sedimentation rates and electrocardiograms were taken. In the earlier part of our study, the standard methods for determining prothrombin time and platelet counts were done in a few cases, but were discontinued because our resources did not permit. Nearly all studies were carried out in the late fall, winter, and early spring.

In our controls were 7 "normal" people who were placed in bed at absolute bed rest on hospital entry for varying conditions, i.e., 3 for psychoneurosis, 1 each for Little's disease, acute gastritis, thoracic aneurysm, and diaphragmatic hernia. Patients with major infections, surgical operations, and hemorrhage were purposely excluded, since Waugh and Ruddick have shown accelerated coagulation in a series of such cases (14). All had normal cardiac findings. Seven other cases had hypertension and arteriosclerotic heart disease with congestive failure, all on digitalis. Two other cases had severe angina pectoris, with 1 on digitalis. The 9 "cardiacs" were on absolute bed rest from the time of admission until observations were completed. Previous to hospital entry, the "normal" group had been ambulatory most of the day, and the cardiac control group was so at least part of the day before hospitalization. The patients with coronary thrombosis had also been ambulatory until the onset of acute symptoms. Patients taking digitalis were included in our control observations because 6 cases of coronary thrombosis were also taking digitalis.

All 27 cases of coronary thrombosis were diagnosed by the usual clinical and electrocardiographic evidence, and were treated by accepted therapeutic measures. Coagulation studies, using the Waugh-Ruddick technique (13), were made as early as possible from the date of entry, and were continued until discharge or death of the patient. At first the observations were made every day. Later, when it became apparent that this was unnecessary, tests were run 2 to 3 times weekly for 4 weeks, then weekly until discharge. More frequent observations were made during the 2nd and 3rd week and when complications ensued. In several instances, control observations were made

months after discharge. In these few instances there was little difference in the coagulation studies on discharge after recovery, from those taken on the same patient months later. Ten of the 27 cases were observed at the Jewish Hospital.[3]

In all but 2 cases the exact date of onset of occlusion could be fixed on clinical and electrocardiographic evidences. The error in these 2 cases is a matter of 1 to 2 days. In several cases, the first observations were not made until 4 or 5 days after the thrombotic episode, usually because the patient had not entered the hospital. The majority of cases were observed within the first week of the initial attack. All questionable cases of coronary occlusion were excluded from our series. One case after discharge, on resuming her duties, developed another attack and was counted as a new patient.

METHOD OF PLOTTING AND EVALUATION

In the Waugh-Ruddick test (13) in order to recognize the phenomenon of accelerated coagulability of blood, controlled deceleration of the process with heparin in "slow motion" magnifies finer changes which can be more accurately measured. In brief, this test consists of recording the coagulation time of the blood in a series of 9 tubes, to which increasing quantities of heparin are added, A curve is then constructed, using the coagulation time in minutes on the ordinate, and the tubes with varying concentration of heparin on the abscissa. When it became apparent to us that there were distinct differences in the clotting curves following a thrombotic attack (Figure 1), the statistical significance was next considered. For this purpose the coagulation end-point of Tube 9 containing $\frac{7}{10}$ unit of heparin was selected, because here the "slow motion" is the slowest, and hence can be read with the least error as we practiced the technique of Waugh and Ruddick. Figure 1 illustrates a typical series of curves obtained during the course of the disease in 1 patient. The smaller the figures in minutes (i.e. Tube 9) the greater the tendency toward acceleration of the clotting tendency. This is spoken of as "flattening of the curve" as acceleration develops. Tube 9 is used in making Figures 2 to 4, and in evaluating results. However, it is evident that the other tubes bear a systematic relationship to Tube 9 in terms of coagulation times. Tube 5 or 6 might well be chosen, but the differences are not as great as in Tube 9 where there is a greater concentration of heparin.

Realizing that certain difficulties may be experienced in obtaining samples of blood, since trauma incident to venous puncture may increase the thromboplastin in a particular sample to be examined, certain precautions were observed as suggested by the authors of the method. A large gauge (No. 18) needle was used, the blood being expelled through the needle. At least 15 ml. of blood
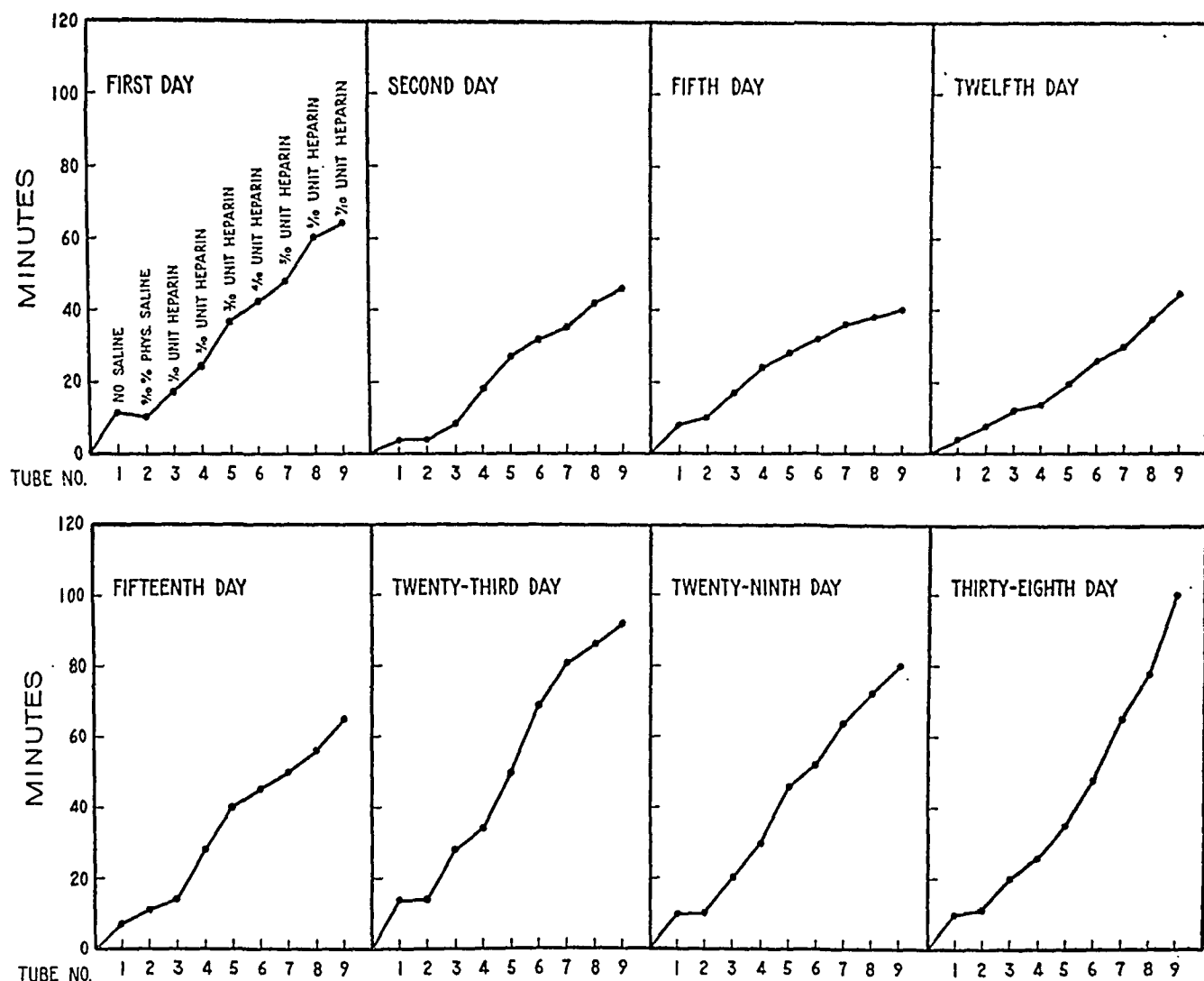
PATIENT: G.C.

FIG. 1. TYPICAL CHANGES IN BLOOD COAGULATION IN A CASE OF CORONARY THROMBOSIS

were utilized for each test. The blood was not used for the determinations if less than this amount of blood was obtained. We were particularly careful to prevent foaming of the blood since this altered the results. The glassware used was new, and especially prepared as suggested by Waugh and Ruddick. All samples of blood were drawn and tested by two of the authors of the present paper in order to insure uniformity of observation.

The controls

In our control group the 7 "normals" and 9 "cardiacs" differed only as to degrees of elevation initially and in the 3rd day in bed (see Figure 2). The general shape of the control curves are similar except that a more precipitous drop occurred with the cardiacs over the 3rd day to the 9th day. Nearly all of our controls showed mild flattening with acceleration of the curves from the 3rd day

on. Four cases taking digitalis dipped into the zone we arbitrarily called acceleration of coagulation, such as occurred so commonly when there was coronary thrombosis. We do not know the significance of this, nor do we see any particular effect of digitalis therapy in the patients with coronary thrombosis who were also taking digitalis.

Coronary thrombosis

Out of the total of 27 cases, 21 (77.8 per cent) showed the phenomenon of accelerated coagulability during some point early in the hospital stay, and usually before the 12th day after the occlusion in an individual case. Very commonly, acceleration occurred by the 3rd day, and remained accelerated from several days to weeks. On a

composite graph (Figure 2) the greatest dip seemed to occur on the 13th day. In one case, acceleration remained as late as the 32nd day. The majority of cases, with the exception of 4, returned to normal by the end of the 3rd week. One case, who showed acceleration for a longer period than the others, after returning to normal, developed another attack on his 37th day after his
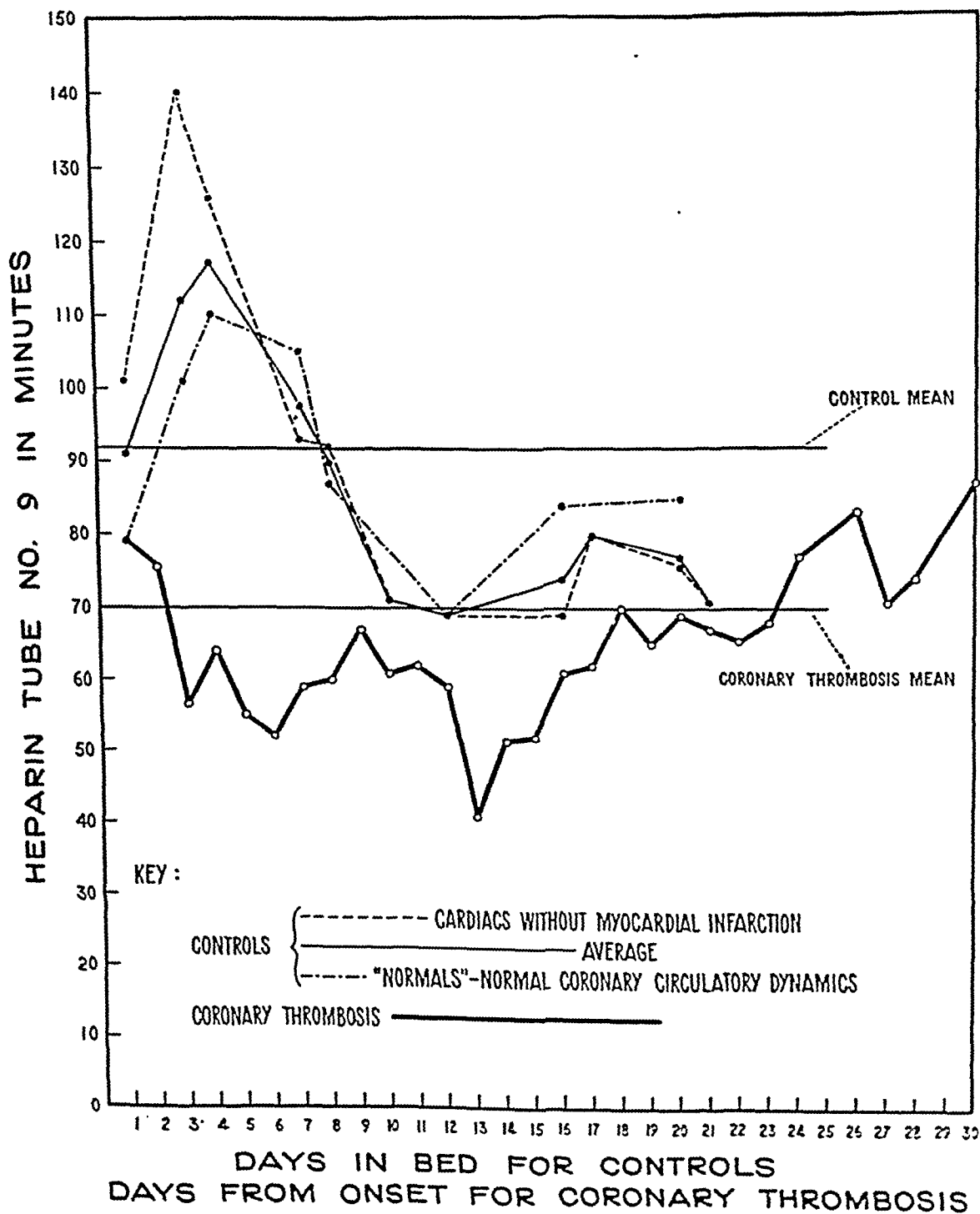

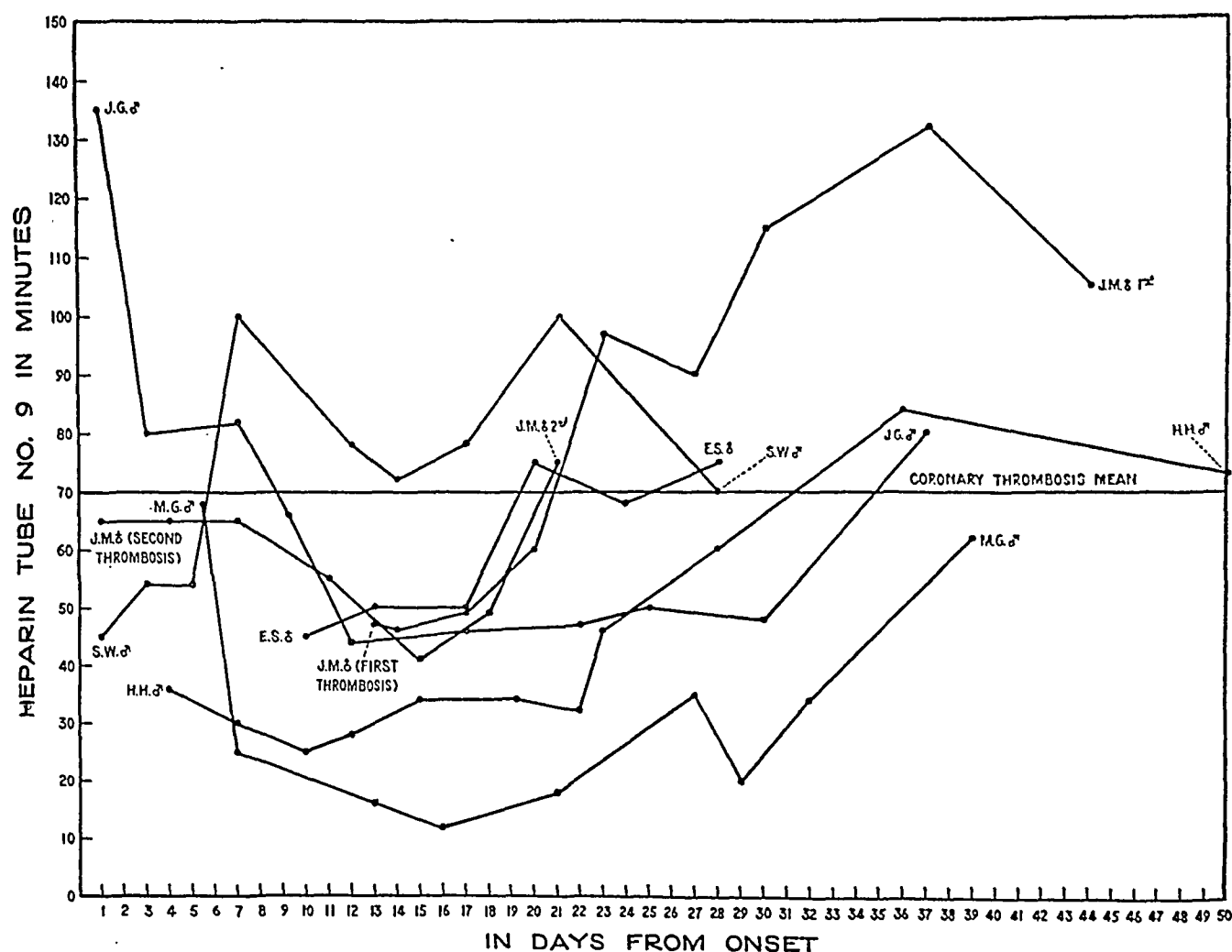FIG. 2. CHANGES OF BLOOD COAGULATION IN CONTROLS AND CORONARY THROMBOSIS

FIG. 3. CHANGES IN BLOOD COAGULATION IN SEVERAL UNCOMPLICATED CORONARY THROMBOSIS CASES

first episode and again showed acceleration (Figure 4, D.B., male). Two were accelerated for a period of a few days beyond the 3rd week. The last case did not show acceleration until the 12th day after the attack, and consequently returned to normal at a later date than usual.

In order than one may visualize changes that occur in an individual case (uncomplicated coronary thrombosis), the values for Tube 9 have been plotted in serial fashion for several patients (Figure 3).

The nature of coronary heart disease did not make it seem feasible to test all the patients every day. On the other hand, we do not think the curve would be materially changed by daily testing. The points on the curves for coronary thrombosis in Figure 2 are the average values of Tube 9 for each day after onset in all of the 27 cases. The number of patients tested on each day varies from 8 to 14, the greatest number being read from the 7th

to the 20th day. Our data appeared valid when analyzed statistically.[4] The data were analyzed

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[4] *Statistical analysis.*

If our results are analyzed statistically, we find the following:

Mean control ....92.2 mean minutes
Mean coronary thrombosis ....70.3 mean minutes
Standard deviation control ....24.58 minutes
    Coronary ....25.16 minutes
Probable error control ....plus 2.34 minutes
    Coronary ....plus 1.16 minutes
Coefficient of variation control 26.64
    Coronary 35.9
Probable error difference ....2.67 minutes

The difference between the coronary group and the control group with reference to mean time for coagulation of Tube 9 on the heparin scale is 21.9 minutes. The probable error of this difference is plus 2.67 minutes. Using the threshold of 4 times the probable error for statistical significance, this difference is seen to be markedly significant, since the difference is 8 times its own probable

by Dr. T. J. Le Blanc,[5] Professor of Preventive Medicine, University of Cincinnati, College of Medicine.

OTHER CLOTTING METHODS

In the individual experiments, the Lee-White and the capillary clotting times run simultaneously with the Waugh-Ruddick test failed to reveal any strikingly significant change when the latter test was accelerated or normal. From our controls (Figure 2) one is struck by the majority of cases being above 60 minutes in Tube 9 of the Waugh-Ruddick test.

To compare the Lee-White and the capillary tube methods with Waugh-Ruddick method, we chose 60 minutes (for Tube 9) as the dividing line above which we considered normal and prolonged, and below which we considered it accelerated. When our Lee-White and capillary results were divided according to the above selection and our findings compared, the following was revealed:

	L. anb W.	Cap.
Waugh-Ruddick normal or prolonged	9.45 minutes	4.05 minutes
Waugh-Ruddick accelerated	8.75 minutes	3.35 minutes
Difference	0.7 minute	0.7 minute

While this difference may not seem significant, it is important that the average time was actually

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error, meaning that the odds against a deviation as great or greater than this are about 14 million to one.

As to variability (the "two universes"), at first glance it might appear that the coronary cases show much greater variability than the controls. While the variability in this group is greater, it is not as much as would appear on inspection. The two standard deviations are relatively close together, and when these are expressed as coefficients of variability the respective values are 26.64 for the controls and 35.9 for the coronary group. In other words, the high variability inherent in the control group does not have much significance, and it is doubtful that any clinical interpretation may be made of such a difference.

It is recognized that the respective variables are high (standard deviations), but in spite of these high levels which enter into the respective probable errors of the means, the probable error of the difference between the two means is either sufficiently low, or the difference sufficiently high, to make this difference strongly implied. (See R. Pearl, "Introduction to Medical Biometry and Statistics," Ed. 2, 1930, P. 283, W. B. Saunders Co.)

[5] Dr. T. J. Le Blanc, Professor of Preventive Medicine, University of Cincinnati, College of Medicine, kindly aided us in the statistical analysis.

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lower for both the Lee-White and capillary clotting times when acceleration was present in the Waugh-Ruddick test. It should be stated, however, that the final interpretation of the results was based on the Waugh-Ruddick test, rather than any other method.

A few platelet counts and a few standard prothrombin times were done, but we did not employ this method enough to determine if accelerated clotting could be demonstrated.

An attempt was made to correlate trends of acceleration of coagulation with the erythrocyte sedimentation rate, but no clear cut relation could be established. Generally, acceleration returned to normal before the sedimentation rate did. Within the first 48 hours of the attack when an increase of the sedimentation rate was present, acceleration may be present. Later, when acceleration usually occurred, the sedimentation rate was almost always increased. After the 3rd or 4th week, when there was no demonstrable acceleration, the sedimentation rate often was still increased.

RELATION OF COMPLICATIONS TO ACCELERATION

Figure 4 shows a random selection of cases who developed complications of coronary thrombosis while being observed.

Patient, J. P., male, had a recent anterior myocardial infarct on admission. Progressive tendency to acceleration of coagulation developed. On the 5th day after the initial episode, the patient developed another clinical attack, and the electrocardiogram revealed evidences of a posterior infarction. The curve approached normal on the 21st day, and no further complications were noticed.

Patient, R. B., male, had a recent posterior myocardial infarction on admission. A sharp tendency to acceleration developed. On the 6th day after the onset another attack occurred, and the following day the electrocardiogram revealed an anterior infarction.

Patient, R. B., female, showed a recent anterior myocardial infarction on admission. A flat curve was present when on the 12th day after onset, patient developed a pulmonary embolus and the following day a shift to R.A.D. in the electrocardiogram, with clinical evidences of pulmonary infarction. The curves thereafter returned to normal on the 29th day. A few days after discharge, the patient developed another new coronary thrombosis.

Patient, J. G., male, revealed an anterior infarct on entry. Progressive flattening developed. When maximum acceleration was present, patient developed more evidences of posterior wall involvement.
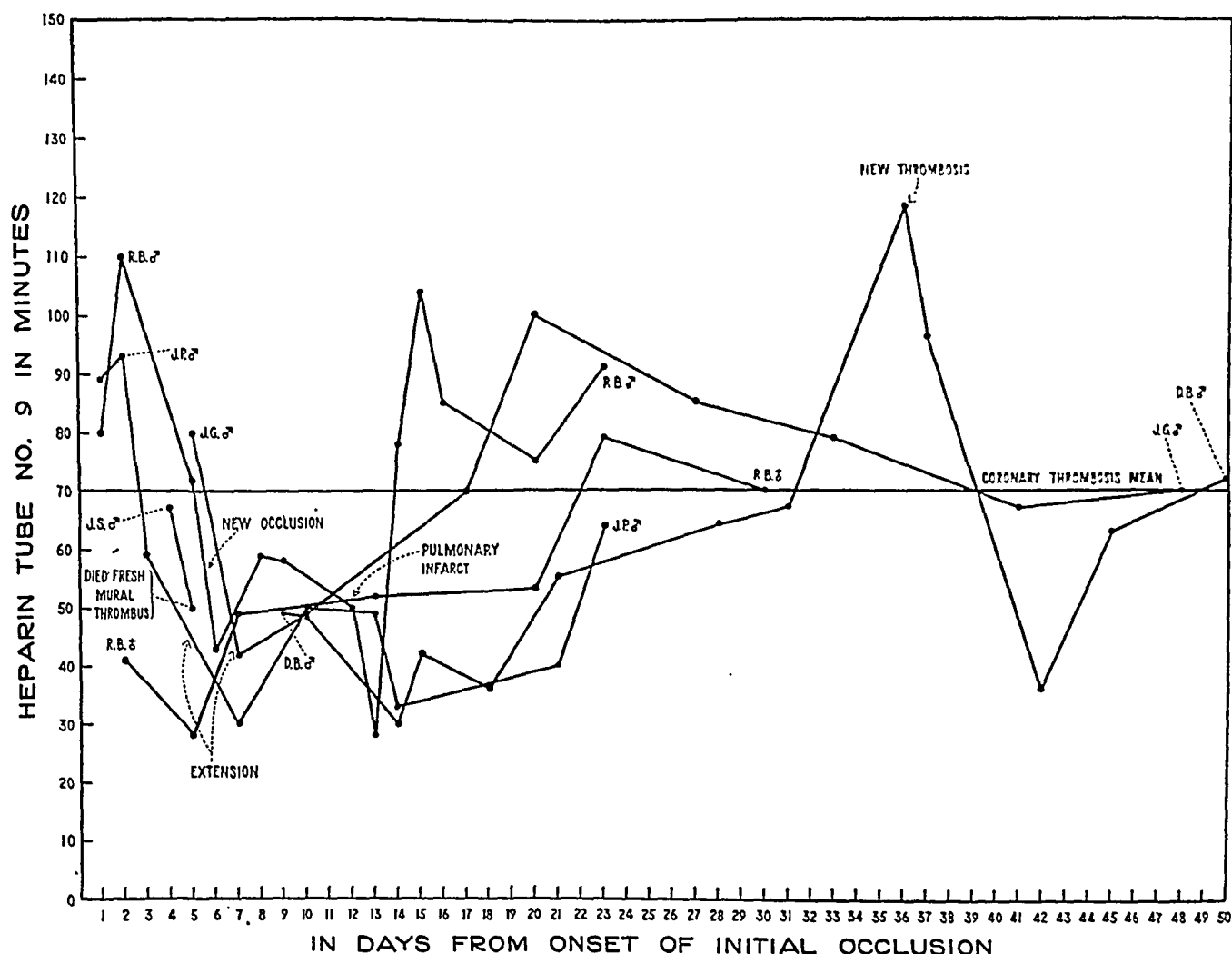
FIG. 4. CHANGES IN BLOOD COAGULATION IN COMPLICATED CASES OF CORONARY THROMBOSIS

Patient, J. S., male, presented an interesting picture. A history of dull precordial pain 3 days before hospital admission was obtained. Patient also had diabetes mellitus. Because of left-sided failure, the patient was placed on digitalis. Electrocardiograms revealed an anterior infarction. The coronary occlusion only contributed to the patient's exitus, inasmuch as it was a small apical infarct (no coronary thrombosis, but moderately severe coronary atherosclerosis of the left anterior descending vessel was present). Of major importance was the fact that the patient died of uremia due to Kimmelstiel-Wilson's disease. Acute fibrinous pericarditis was also present. This patient was included in this group since a large very fresh mural thrombosis of only several days' duration was found.

Patient, D. B., male, who previous to admission had an old anterior infarction, developed a new posterior infarct responsible for his admission. Acceleration observed immediately on admission was maintained for 31 days before returning to normal. On the 36th day after the onset of the posterior infarct, another attack developed with new electrocardiographic findings (extension) with progressive tendency towards acceleration. Seven days later maximum flattening occurred.

The first 4 cases would suggest that when the phenomenon of acceleration was present, the patient was liable to a new thrombosis or to extension or to thrombo-embolization. The curve of patient D. B., male, suggests that after returning to normal, the initiating mechanism of the new occlusion or thrombosis is not necessarily dependent on the coagulatory mechanism. However, the phenomenon of acceleration following occlusion is again demonstrated.

Five cases of coronary thrombosis died while in the hospital. Two died of acute heart failure, 3 died of coronary thrombosis or massive pulmonary embolus. All except 1 showed acceleration at some time. Two days before death, curves were normal in 3. One showed acceleration continuously until death. One died in the 4th day of onset without showing acceleration. Two cases came to autopsy while under study, and one only showed mural thrombus.

DIGITALIS, XANTHINES, AND CORONARY THROMBOSIS

It has recently been claimed that digitalis (16 to 19) and the xanthine (20) drugs favor accelerated coagulation, and therefore due regard was given to that point in our study. It would appear from our control group who required it, that as the patients were digitalized some acceleration develops.

Of the 6 patients with coronary thrombosis taking digitalis (Figure 5), 4 were on maintenance doses when they developed their occlusion, and 2 were digitalized on hospital entry after occlusion.

Of the 6, 2 cases (J. S. and N. P.) revealed acceleration curves that differed from our usual type in neither degree of acceleration nor duration. Both cases died, however.

Three of the remaining 4 on maintenance doses of digitalis returned to normal after the usual acceleration, showing no continued acceleration even though digitalis continued to be taken.

The remaining case (K. P.) was not observed until late in the 2nd week. No acceleration was found during her entire stay.

Again, these cases are too few from which to draw any conclusions. One may say that the accelerating response following coronary occlusion would seem to follow its usual course, despite digitalis. If digitalis has an additional accelerating effect, more cases and further studies are necessary to demonstrate the degree of such addition.

Four of the patients with coronary thrombosis received adequate doses of the xanthines during their hospitalization for a period of 3 or more days. None showed any more acceleration, as measured by the Waugh-Ruddick method, than would be expected from coronary thrombosis alone.
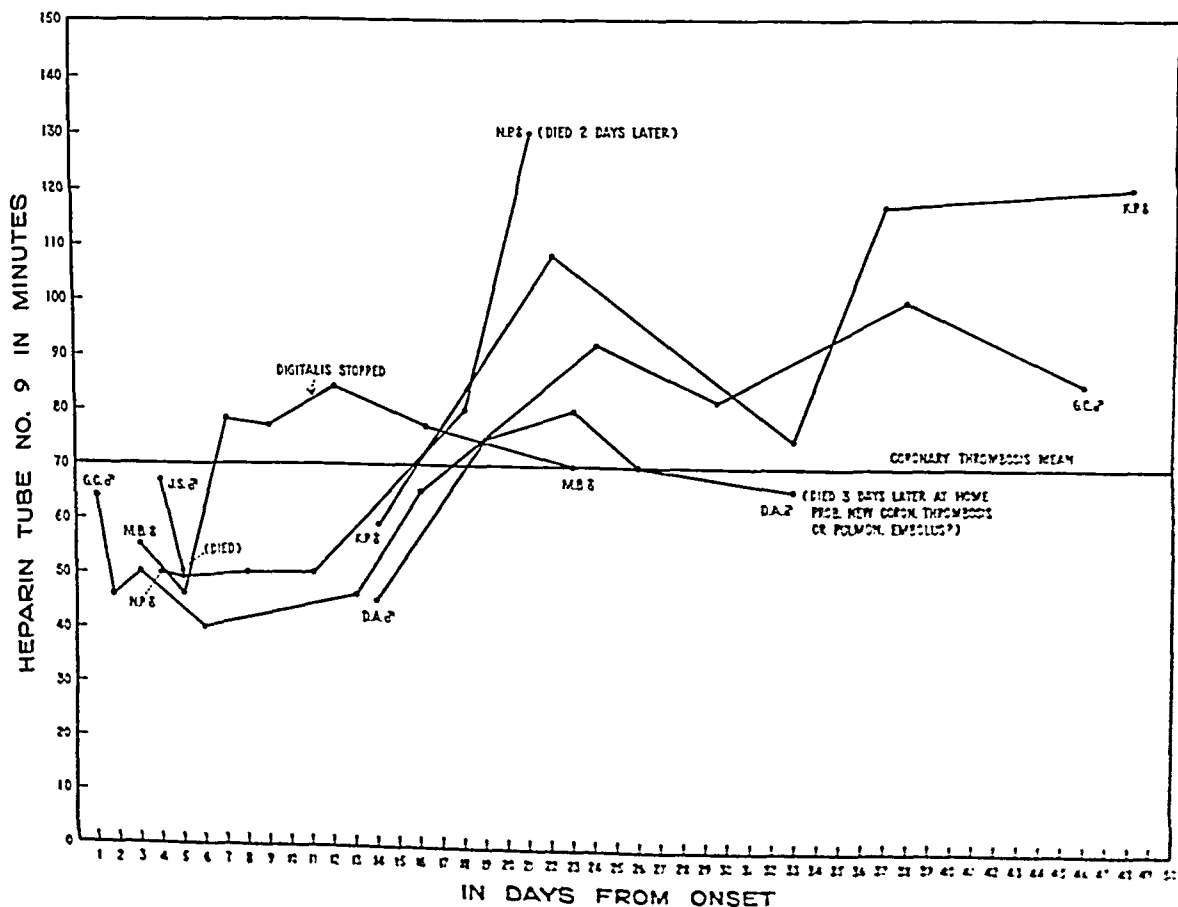
Three patients from the cardiac control group

FIG. 5. CHANGES IN BLOOD COAGULATION IN CORONARY THROMBOSIS CASES ON DIGITALIS

were also given large doses of xanthines: 2 received aminophylline intravenously, and 1 received theobromine in large doses. No acceleration was noted, other than what was expected from bed rest alone.

No conclusions can be drawn from such a small series of cases, although Field *et al.* (20) have demonstrated hyperprothombinemia in animals receiving xanthine drugs. We do not know if any parallel can be drawn between their methods and the Waugh-Ruddick technique.

DISCUSSION

It is admitted that the Waugh-Ruddick test is empirical, artificial and may not represent what occurs *in vivo;* the same may be said for other coagulation tests. When we compare the Lee-White and capillary tube methods with the Waugh-Ruddick tests, we find that both of the former tests reveal a faster clotting time by 0.7 minute when acceleration by the Waugh-Ruddick method was present. This difference does not look very significant. However, when both tests were on the average faster, this assumes more significance than if only one were faster. Whether it is this small difference we are measuring on the Waugh-Ruddick test remains to be seen.

In reality, the Waugh-Ruddick test is an *in vitro* De Takats Heparin Tolerance Test. The "hyporeactors" which De Takats (1) found in various thrombotic states would be consistent with our findings.

Since we are employing a new method in studying the coagulation of the blood, a discussion of the method seems in order. It is true that by the addition of heparin, small differences are magnified. However, the differences are also evident in Tubes 3 or 4, where smaller concentrations of heparin are used. Unfortunately, the various methods which have been devised for measuring the clotting time, while satisfactory for demonstrating a prolongation of the process, are difficult to interpret when acceleration occurs. This is probably due to the fact that, under normal conditions, the coagulation time is relatively short, and unless conditions are very carefully controlled, acceleration of coagulation may be very difficult to detect.

As we have gained experience with the method

we have noticed a remarkable consistency in the determinations from day to day. This is especially evident in the ambulatory patients. In the control group, bed rest alone causes some acceleration of the curve (Figure 2). After this initial acceleration, which reaches its maximum about the 9th day, the curves remain more or less stabilized. In the coronary group, as the curves later approach normal, they are quite similiar to the curves taken on the first day of the episode. All their observations suggest that chance alone does not determine the results. Our findings in the control group on rest alone, and in the ambulatory patients, are in accord with those of the authors of the method (14). In considering the significance of the method, we are probably measuring the concentration of thromboplastic substances in the blood. Brinkhous, Smith, Warren, and Seegers (21) have demonstrated that heparin along with its serum complement is antagonistic to thromboplastin.

Waugh and Ruddick (14) believe that the flattening of the curve is due to an increase in thromboplastin. They have shown that by increasing the concentration of thromboplastin, a flattening of the curve results. Likewise, removal of platelets from the blood by centrifuging, markedly prolongs the coagulation of the blood in their test. If we consider that thromboplastin could easily come from infarcted damaged myocardium, or from disintegrating platelets which initiate mural thrombus formation, this explanation becomes even more attractive. Thromboplastin, although present in the platelets, can be derived from many tissues, and hence, is essentially a tissue extract. It is probable that the increased thromboplastin present in the blood following coronary thrombosis and myocardial injury is in no way different from that which occurs following hemorrhage, muscle injury, trauma, or surgical or obstetrical intervention. An added fact which supports this thesis is that marked acceleration of coagulation in the cases of coronary thrombosis is not observed immediately after the accident, but 2 to 3 days later, after myocardial tissue damage has resulted from the thrombosis, and the mural thrombus begins to form. Hence, accelerated coagulation probably plays no rôle in the causation of the thrombosis, but is rather the result of the thrombosis and its subsequent tissue damage. However,

once acceleration is present, it may possibly lead to further complications.

Until some method of isolating, or more positively, demonstrating, thromboplastic alteration of the blood is developed, further comment would only be superfluous. Whether the acceleration of "prothrombin time" reported by Shapiro (4) in the 5 cases of thrombo-embolization would occur in coronary thrombosis will require further study. Doles (7), however, found in 13 cases of coronary thrombosis, using Smith's whole blood method for prothrombin determination, that after an occlusion the prothrombin time was reduced to an average of about 57 per cent of normal 12 to 48 hours after the attack. Consequently, he used vitamin K in treating his cases. Quick (22) has several serious objections to the Smith Bedside test. Although Doles' (7) studies were on coronary thrombosis, his findings would seem to vary from others who have found accelerated "prothrombin times." It should again be emphasized that the acceleration of coagulation found in coronary thrombosis is not specific, since it is found in thrombosis and embolus elsewhere, in postoperative patients following hemorrhage, acute infection, or childbirth.

Our groups of 27 hospital observations on coronary thrombosis is by no means a large series upon which to draw any definite conclusions. The findings suggest that following a thrombotic or occlusive episode a tendency to acceleration develops. An increasing tendency towards acceleration occurs reaching an initial low level by the 3rd day, and remaining relatively low, to reach an apparent maximum dip by the 14th day. A general maintenance of acceleration seems evident until the 17th day. Thereafter, the curve becomes indistinguishable from the control group. The observations on the first day of these 27 patients and the control group seem similar. It may be said that the blood coagulative mechanism shows little alteration from normal the first day after the attack, and from the 17th day onward, by this method. This would suggest that the clotting mechanism preceding the attack, and after the 3rd week does not differ from the normal. The occurrence of 5 cases with definitely proven complications, such as a new thrombus in a period in which there is acceleration, suggests that acci-

dents tend to occur when favored by accelerated coagulability of the blood. Clinicians are aware of this dangerous period in recovering from coronary thrombosis.

If future investigations should confirm these findings, the implications are obvious. The dangers of anticoagulant therapy are well known. The liability to retrograde progression of coronary thrombus, and to formation of a new thrombus also, is speculative, but the incidence of mural thrombus and embolization is so high in our experience that some new approach to the problem of therapy is desirable. From unpublished data (12), formation of mural thrombi seemed more frequent 48 hours after the onset of the thrombosis, fewer between 24 and 48 hours after onset, and rarely within the first 24 hours. This would coincide with the more frequent embolization occurring between the 7th and the 10th day of onset. The chances of preventing these complications would seem better the earlier the therapy is commenced.

CONCLUSIONS

Twenty-seven cases of acute coronary thrombosis were studied in hospital to find evidence of accelerated blood coagulation. In 77.8 per cent of the cases, an acceleration as measured by the Waugh-Ruddick test occurred usually by the 2nd or 3rd day, lasting to about the 17th day. Occasionally, acceleration was delayed until the 12th day of the illness, and persisted only for several days. Acceleration was prolonged beyond the 3rd week in a few cases.

Of 16 controls, 9 being in various stages of heart failure, none showed acceleration to the degree seen in coronary thrombosis. The statistical significance of this finding is set forth. An explanation is not attempted.

Such complications as new coronary thrombosis or extension of the original thrombus or pulmonary infarction, while admittedly too few for definite conclusions, developed in 5 patients when acceleration was present, suggesting that this factor may be contributory.

Our findings further suggest that the first attack is not initiated by accelerated coagulability of the blood in the average case, and that after the 4th week the clotting mechanism is indistinguishable from normal.

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ADDITIVE EFFECTS OF IODINE AND THIOUREA IN THE TREATMENT OF HYPERTHYROIDISM [1]

By T. S. DANOWSKI, E. B. MAN, AND A. W. WINKLER

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Evidence has already been presented that prior administration of iodine to patients with hyperthyroidism does not prevent the action of thiourea (1). Some cases behaved as if the action of iodine actually supplemented that of thiourea, but the evidence for such an additive effect was inconclusive. On the other hand, various writers have maintained that preliminary treatment with iodine delays the action of thiouracil (and so, possibly, of thiourea), even if it does not entirely obviate it (2 to 11). From a practical as well as a theoretical viewpoint it is essential to decide which interpretation is correct. If the actions of the two agents are additive, or at least do not interfere with one another, they may well be given together. If, on the other hand, the prior or simultaneous administration of iodine delays the action of thiourea, it would be advisable to avoid combined treatment. The present study seeks to resolve this problem by a comparison of the effects in hyperthyroid patients of treatment with thiourea alone, and with both iodine and thiourea. Further observations on the treatment of hyperthyroidism with thiourea are included, particularly concerning the eventual need for thyroid medication to control hypothyroidism.

MATERIALS AND METHODS

Fifty-four patients in all were studied. Some data in 16 of these have been previously reported. The diagnosis of hyperthyroidism was based primarily upon the presence of a concentration of precipitable iodine in serum of 8 or more gamma per cent (12). Basal metabolic rate initially exceeded + 20 per cent in all but 5 cases. In all cases the history, symptoms and physical findings were compatible with the diagnosis of hyperthyroidism. About 85 per cent of the patients in this series had diffuse enlargement of the thyroid gland, the remainder had toxic

adenomata. Unequivocal exophthalmos was present in one-third of the cases. No attempt was made to assign a particular plan of treatment to patients in any one of these categories. Four regimes were employed in the first few weeks of treatment. Sixteen patients received thiourea alone, either in a single daily dose of 0.28 gram, or in three daily doses of 0.07 gram. Fifteen patients were treated simultaneously with thiourea in similar dosage and with 5 drops of strong solution of iodine (U.S.P.) three times a day. Twenty-three patients were first treated with strong solution of iodine (15 drops daily) for periods ranging from 2 to 52 weeks, and then given thiourea. All but 5 of these patients were incompletely controlled on iodine medication alone, judging from the level of the serum iodine and the clinical status. In 17 of these the iodine was continued along with the thiourea medication, while in the remaining 6 iodine was discontinued as soon as the thiourea medication was begun. With remission of the hyperthyroidism and decline of the serum precipitable iodine to normal or subnormal levels, the dosage of thiourea was either decreased from 0.28 to 0.07 gram or less once daily, or else the patient was given desiccated thyroid, 0.03 to 0.06 gram daily. Administration of thiourea has been continued to date in all but 2 cases. In all patients determinations of the serum precipitable iodine (13, 14), the basal metabolic rate, the body weight, and the pulse rate were repeated at intervals of 2 to 10 weeks. Leukocyte and other blood counts were obtained, as well as urinalyses.

RESULTS

(A) Initial response to treatment. Administration of thiourea alone produced clinical improvement and a drop of the elevated concentration of precipitable iodine in serum to normal within 6 weeks in 11 out of 16 hyperthyroid patients (Figure 1A). Subsequently the serum iodine declined to normal levels in 2 of the 5 patients who failed to respond during the initial 6 weeks of treatment (M. W., C. V.). In 3 other patients (J. C., M. Ma., D. G.) the serum precipitable iodine rose again to hyperthyroid levels following the initial drop with a subsequent return to normal in 2 out of the 3.

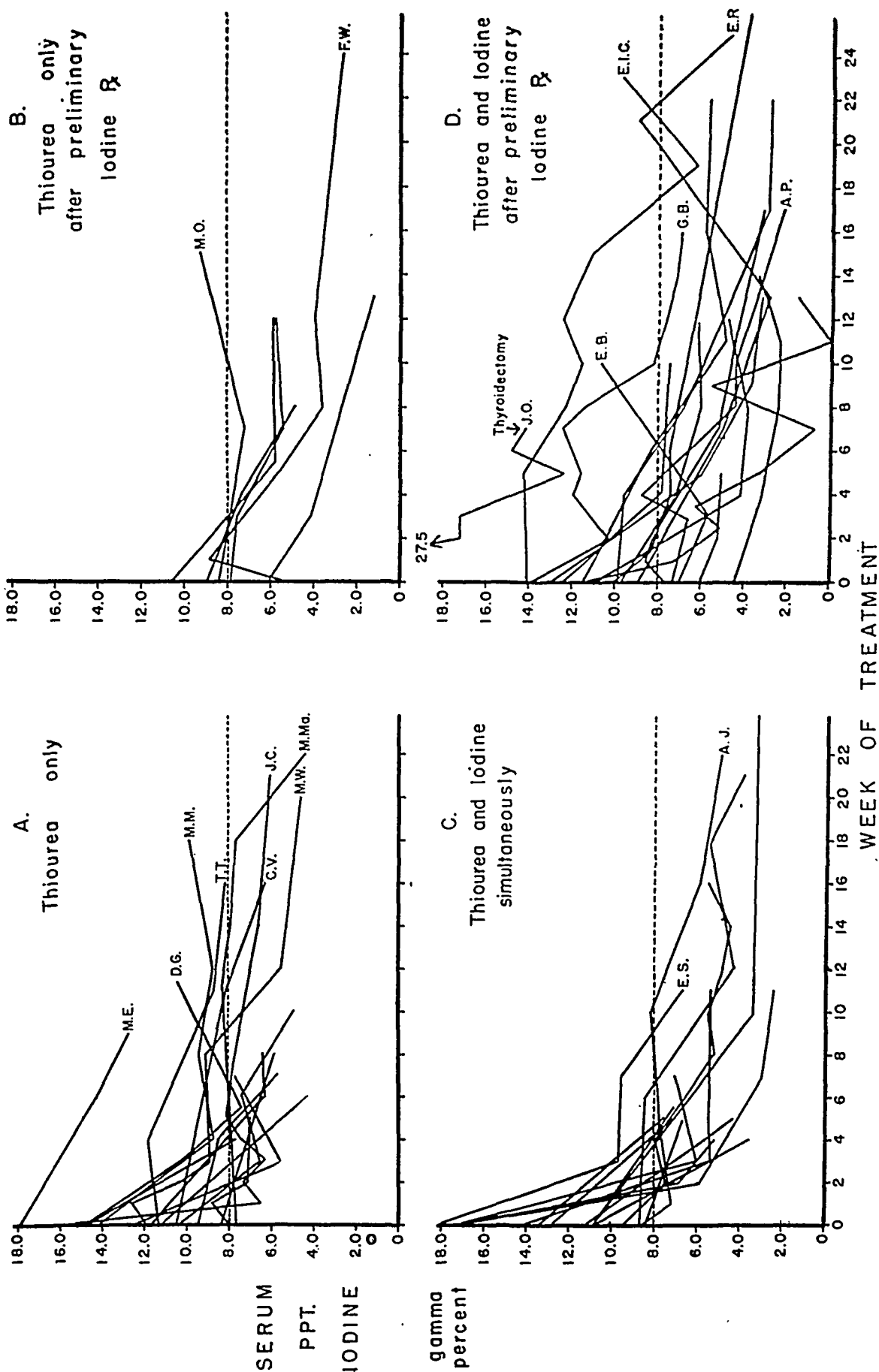A comparison of Figures 1A and 1C proves

TREATMENT OF HYPERTHYROIDISM

FIG. 1. EFFECTS OF IODINE AND THIOUREA IN DIFFERENT COMBINATIONS ON THE SERUM PRECIPITABLE IODINE OF HYPERTHYROID PATIENTS

Values above 8 γ per cent are regularly associated with hyperthyroidism, those below 3 γ per cent with hypothyroidism. Thiourea and iodine simultaneously (C) are somewhat more effective than thiourea alone (A). Preliminary iodine treatment (D) does not, on the average, delay the response to thiourea and iodine.

that iodine administered simultaneously with thiourea in no way delays the effectiveness of the latter. Actually, at the end of 6 weeks of treatment all but 2 of 15 patients had responded with a decrease in serum precipitable iodine to euthyroid concentrations. This result is to be contrasted with that observed in the group treated with thiourea alone, in which at the end of 6 weeks the serum iodine level had not returned to normal in 5 out of 16 patients. Furthermore, from the 6th to the 16th week of treatment, the serum precipitable iodine declined more consistently, and to a greater extent, with combined medication than with thiourea alone.

A comparison of Figure 1A with Figure 1D proves that the serum precipitable iodine did not, in general, fall any faster when thiourea alone was given to otherwise untreated cases (Figure 1A) than when it was given to those previously receiving iodine medication, providing iodine medication was not discontinued (Figure 1D). In only 3 cases (G. B., E. I. C., J. O.) in the group which received a preliminary and concurrent course of iodine the serum iodine remained above normal 6 weeks after thiourea was started, a result as satisfactory as that obtained with thiourea alone. The first patient has since responded; the second has continued to be partially refractory for many months, and the third had a thyroidectomy performed after 7 weeks of treatment. In this patient the serum precipitable iodine decreased from 27.5 to 14.2 gamma per cent before operation. Temporary exacerbations under treatment were observed in 3 other patients (A. P., E. B., E. R.).

Comparison of Figures 1A and 1B is somewhat unsatisfactory because of the paucity of cases in the latter group. In one case of Figure 1B (F. W.) there was a transient relapse following the substitution of thiourea for iodine medication. In another patient (M. O.) thiourea alone produced only a temporary remission. The other 4 declined during the first 6 weeks as fast as did the patients in Figure 1A.

(B) *Later response to treatment.* Normal or subnormal levels of serum precipitable iodine appeared after 4 months with equal frequency in all 4 treatment regimes. Thus the eventual effects of thiourea alone, in the dosage employed, could not be distinguished from those of iodine plus thiourea.

The concentration of precipitable iodine in serum was allowed to decrease to hypothyroid levels in 18 of the 54 patients before the therapeutic regime was altered, while in the others it was permitted to decline only to euthyroid levels. Changes in regime were made one at a time, usually at intervals of several weeks.

(1) *Effect of reduction in dosage of thiourea* (Table I). It was possible gradually to decrease the daily dosage of thiourea with only infrequent exacerbations as a result. In 19 patients the initial daily dose of thiourea (0.28 gram at one time, or 0.21 gram divided into 3 equal portions) was reduced to a single daily maintenance dose of 0.07 gram after 2 to 54 weeks of treatment. In 2 of these patients (E. A. C. and C. S.), the dose was further reduced to 0.04 gram daily. Re-

TABLE I

Effect of reduction in dosage of thiourea, after initial remission of hyperthyroidism, on the serum precipitable iodine and the basal metabolic rate

Patient	Dose treatment			Duration from start of thiourea	Serum precipitable iodine**	Basal metabolic rate**
	Thiourea	Strong solution of iodine	Desiccated thyroid			
	grams	*drops*	*grams*	*weeks*	*gamma per cent*	*per cent*
E.B.	0.28	15	0.06	0 to 48	4.9	+10
	0.07	15	0.03	48 to 54	4.9	− 3
W.B.	0.28	5		0 to 9	3.4	
	0.08	5		9 to 50	5.4	
				50 to 65	6.8	
E.A.C.	0.21*	15		0 to 15	4.2	+ 4
	0.07	15		15 to 25	4.4	−13
	0.04	15		25 to 31	3.5	−20
A.B.	0.21*	15		0 to 2		
		5		2 to 4	4.3	
		5		4 to 12	10.8	
J.C.	0.21*			0 to 30	4.8	+ 3
	0.07			30 to 41	5.0	− 6
M.G.	0.21*			0 to 11	4.8	+ 9
	0.07			11 to 19	6.5	+ 3
B.G.	0.28		0.06	2 to 54	3.1	
	0.07		0.03	54 to 62	5.7	
P.G.	0.28	15		0 to 17	3.0	−25
	0.28	15	0.03	17 to 32	2.9	−23
	0.07	15	0.03	32 to 40	3.9	−25
S.L.	0.28	5	0.03	34 to 51	1.7	− 4
	0.07	5	0.03	51 to 56	4.6	− 8
M.M.	0.28			0 to 50	5.3	−15
	0.07			50 to 62	4.7	− 6

TABLE I—*Continued*

Patient	Daily treatment			Duration from start of thiourea	Serum precipitable iodine**	Basal metabolic rate**
	Thiourea	Strong solution of iodine	Desiccated thyroid			
	grams	*drops*	*grams*	*weeks*	*gamma per cent*	*per cent*
M.S.	0.28			7 to 26	6.5	+19
	0.07		0.06	26 to 41	9.3	+37
	0.28		0.06	41 to 46	7.4	+27
	0.07		0.03	46 to 63	4.6	
C.S.	0.21*	15		0 to 8	2.4	−19
	0.07	15		8 to 11	2.4	−26
	0.04	15		11 to 14	3.6	−24
F.W.	0.21*			0 to 24	2.7	
	0.07			24 to 34	4.9	
M.W.	0.28			0 to 26	4.6	−15
	0.07			26 to 32	4.6	−18
M.P.	0.28		0.06	0 to 52	4.1	+10
	0.07		0.06	52 to 59	3.9	+ 8
J.H.	0.28	15	0.06	0 to 71	3.9	− 1
	0.07	15	0.03	71 to 81	4.5	− 7
M.K.	0.28		0.06	0 to 50	2.9	− 3
	0.07		0.03	50 to 60	5.9	+16
F.M.	0.21*	5		0 to 13	2.4	−23
	0.21*	5	0.03	13 to 20	5.1	+23
	0.07			20 to 30	5.5	+19
M.A.	0.21*	15		0 to 4	7.1	−13
	0.07	15		4 to 12	6.3	− 8

* Divided into three equal doses.
** At the end of the period.
† Serum precipitable iodine always exceeded 8.0 gamma per cent at the start of thiourea medication (zero day). The iodine concentration in patient B.G. at 2 weeks was 6.1, in patient S.L. at 34 weeks 5.0, and in patient M.S. 5.1 gamma per cent at 7 weeks.

duction of dosage was followed by an exacerbation of hyperthyroidism in one patient (M. S.) who had previously been controlled for 26 weeks with 0.28 gram of thiourea daily. Resumption of this dosage again induced a remission. The hyperthyroidism has remained in abeyance with the smaller dosage of thiourea in the other 18 patients. They have now been followed for periods of time ranging from 4 to 41 weeks.

Thiourea has been discontinued in 2 patients (W. B. and A. B.), after 50 weeks and 2 weeks of thiourea treatment respectively. Patient W. B. has remained well to date, 15 weeks following the withdrawal of thiourea, although iodine therapy has been continued; the second patient (A. B.) suffered a relapse within 10 weeks.

(2) *Effect of omission of the strong solution of iodine* (Figure 2). Iodine solution was omitted after varying intervals of treatment (1 to 31 weeks) in 6 patients in whom thyroid overactivity was completely controlled by simultaneous administration of thiourea and strong solution of iodine. In 4 of the 6 patients serum precipitable iodine and basal metabolic rate promptly rose to hyperthyroid levels. There was also a recurrence of the symptoms of hyperthyroidism. These changes were present at 5 to 8 weeks following withdrawal of the iodine solution, and, judging from the patient's histories, may well have developed earlier. Two of the 4 patients (B. P. and W. B.) were again given iodine as well as thiourea with prompt disappearance of all signs and symp-

TABLE II

Effect of treatment with desiccated thyroid on the serum precipitable iodine of patients, previously hyperthyroid, who had become euthyroid or hypothyroid under treatment with thiourea

Patient	Daily treatment			Duration from start of thiourea	Serum precipitable iodine*	Basal metabolic rate*
	Thiourea	Strong solution of iodine	Desiccated thyroid			
	grams	*drops*	*grams*	*weeks*	*gamma per cent*	*per cent*
E.B.	0.28	15		0 to 12	4.8	− 9
	0.28	15	0.06	12 to 48	4.9	+10
B.G.	0.28			0 to 19	1.3	
	0.28		0.06	19 to 32	4.0	
	0.28		0.06	32 to 54	3.1	
P.G.	0.20	15		0 to 17	3.0	−25
	0.20		0.03	17 to 32	2.9	−23
J.H.	0.20	15		25 to 29	0.5	+10
	0.20	15	0.06	29 to 67	4.2	− 1
M.K.	0.20			1 to 12	5.8	+11
	0.20		0.06	12 to 50	2.9	− 3
S.L.	0.20	15		0 to 23	2.9	−19
	0.20	15	0.06	23 to 28	6.3	+36
	0.20	15	0.03	28 to 51	1.7	− 4
B.P.	0.07	10		0 to 13	3.2	−14
	0.07	10	0.06	13 to 26	7.0	+ 1
A.P.	0.14	5		0 to 17	2.2	− 6
	0.14	5	0.03	17 to 39	4.3	−15
M.P.	0.28			0 to 14	2.9	+10
	0.28		0.06	14 to 52	4.1	+10
M.S.	0.28			0 to 20	0.6	+17
	0.28		0.06	20 to 32	6.4	

* At the end of the period.

toms of hyperthyroidism. One of the other 2 patients (A. L.) was again controlled by increasing the dose of thiourea without the use of iodine solution.

Both patients in whom omission of the iodine solution was not followed by a recurrence of hyperthyroidism ultimately developed the low concentrations of serum precipitable iodine characteristic of hypothyroidism.

In 2 cases shown in Figure 2 (B. P. and A. P.) the iodine present in the desiccated thyroid which they were receiving did not prevent a recurrence of hyperthyroidism when the strong solution of iodine was discontinued.

(3) *Effect of desiccated thyroid.* Eight patients received 0.03 to 0.06 gram of desiccated thyroid, U.S.P., following the development of hypothyroid levels of serum precipitable iodine (3.2 to 0.6 gamma per cent) (Table II). In 2 other patients (E. B. and M. K.) thyroid therapy was started before the serum precipitable iodine had

decreased to abnormally low concentration. The response to thyroid medication was most irregular. In some patients even the larger dose of dried thyroid, 0.06 gram daily, did not suffice to restore the serum precipitable iodine, or, in one patient (M. K.) to prevent a further decline. The smaller dose, 0.03 gram daily, proved inadequate in 2 of the 3 patients to whom it was given.

(C) *Toxic reactions.* Two patients developed drug fever up to 103° necessitating withdrawal of the thiourea. The treatment period, 7 and 10 days respectively, was too brief to affect the hyperthyroidism, and these 2 patients are not included in this series.

There have been no skin eruptions. Urine analyses and complete blood counts have remained within normal limits in all patients.

In occasional patients malaise and nausea were present at the start of treatment. These symptoms usually disappeared either spontaneously, or fol-
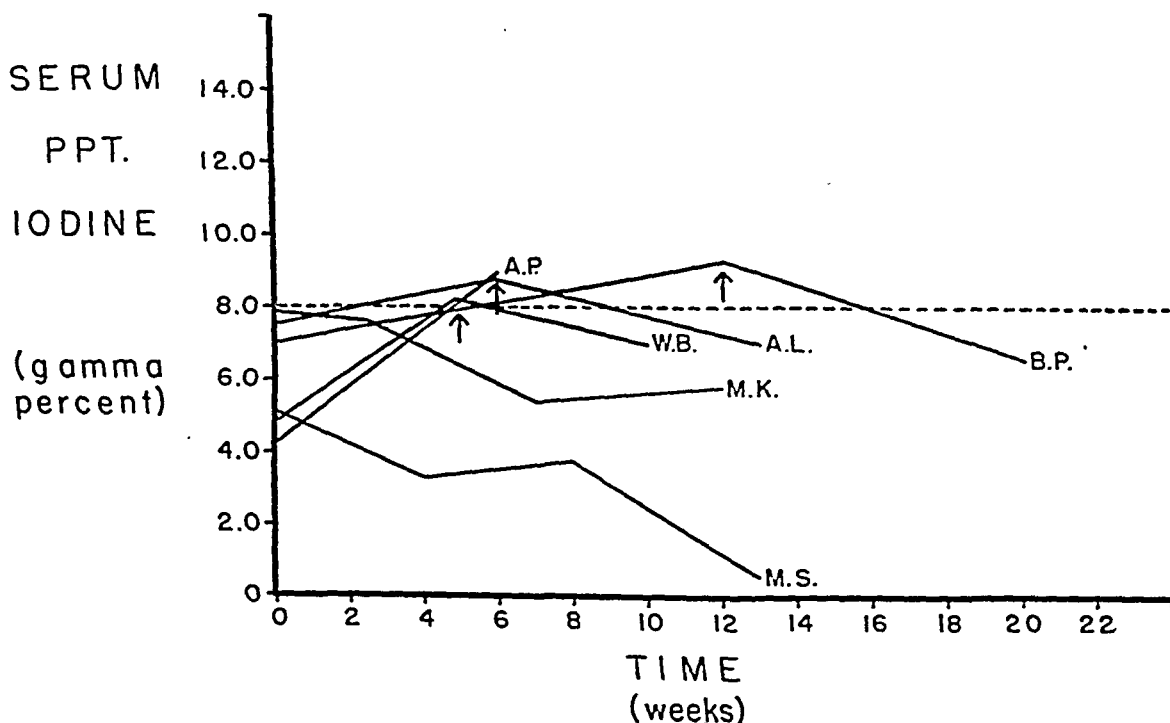

FIG. 2. EFFECT OF WITHDRAWAL OF IODINE SOLUTION IN PATIENTS IN REMISSION ON IODINE AND THIOUREA

Withdrawal of iodine medication in patients in remission on combined iodine and thiourea therapy resulted in a rise in the serum precipitable iodine in 4 of 6 instances (A. P., W. B., A. L., and B. P.). Resumption of iodine medication at points indicated by arrows in patients W. B. and B. P. again produced a remission. A similar effect was produced by doubling the dosage of thiourea, at the point indicated by the arrow, in patient A. L. This is proof of continued additive effects of iodine and thiourea medication.

lowing temporary reduction in the dosage of thiourea.

(D) *Relation between changes in serum precipitable iodine and the basal metabolic rate.* Changes in these two measurements tended to parallel one another, but the fall in serum iodine often preceded that of the basal metabolic rate. The converse was never true. Thus, in 34 cases in which there were sufficient data, the fall of the serum iodine from supranormal to normal levels preceded that of the metabolic rate in 15 instances while in 19 no lag was detected. The lag of the metabolic rate behind the serum iodine was even more evident when the latter fell to subnormal levels, since it was present in 5 of the 7 cases studied.

DISCUSSION

These experiments furnish strong evidence against any theory that the action of iodine in hyperthyroidism antagonizes that of thiourea. They do not support the common opinion that preliminary medication with iodine delays the action of the thio-drugs in the hyperthyroid patient, whatever may be their relationship in the normal rat (15). There is, on the other hand, considerable evidence that the actions of the two substances are additive. The relapse of the hyperthyroidism when iodine alone was discontinued in patients controlled with a combination of iodine and thiourea (Figure 2) is almost conclusive. These relapses are all the more significant since the trend of the serum precipitable iodine is generally downward as thiourea medication is continued over a long period of time. The quickest and most pronounced remissions occurred in patients who had received and continued to receive iodine. Indeed, from a comparison of Figures 1B and 1D, it seems quite probable that the widespread belief that preliminary iodine medication delays the action of thiouracil and thiourea may be related to the practice of discontinuing the iodine at the time these drugs are started. This procedure might superimpose a relapse due to release from iodine effect upon the inhibition due to the thio-drug, with consequent delay in remission. The argument that excess thyroglobulin stored during the preliminary period of iodine administration might cause a delay in the response to thiourea because of the time required for its

degradation, has never been entirely satisfactory. No hyperthyroidism follows the discontinuance of iodine therapy in euthyroid subjects, although extra iodoprotein is stored in the gland and is later destroyed (16). The rapid response of patients on iodine solution and thiourea cannot be ascribed to iodine alone, since almost all of the patients failed to develop a complete remission during the preliminary course of iodine. Furthermore, although iodine solution may in a small percentage of cases produce a return of serum precipitable iodine to normal limits, a depression of the serum precipitable iodine to hypothyroid levels with this agent alone would be almost unprecedented.

These observations not only should remove all fear of giving iodine prior to or along with thiourea, but provide a positive basis for recommending combined therapy. The recurrence of the hyperthyroidism with omission of iodine medication in patients on combined therapy (Figure 2) is positive proof that the administration of iodine may permit a smaller dose of thiourea to be effective. In view of the potential toxicity of all drugs of this series, the practical advantage from such reduction of dosage is obvious. Since there is no reason to believe that the mode of action of thiourea differs from that of thiouracil or its derivatives, this conclusion can logically be extended to medical treatment with all substances of this class. Combined therapy has, indeed, been introduced as a preoperative measure (3, 7), since hyperplasia and operative bleeding are much reduced. The common practice of using the drugs *seriatim* should be replaced by simultaneous administration throughout the preoperative period, as well as for prolonged medical therapy. Demonstration that iodine and thiourea medication are additive in their effects supports and supplements the work of Rawson and his associates (17), who have examined the thyroid gland histologically during a remission induced by thiouracil medication alone, and again after a course of combined iodine and thiouracil medication. They found that, although the functional hyperthyroidism remained in abeyance, the intense hyperplasia induced by the thiouracil treatment alone had partly resolved after the iodine had been given, even though thiouracil medication was continued.

This points to different and independent inhibitory actions by each agent, which might be additive.

· The progressive decline in thyroid activity as thiourea was continued meant that dosage was often cut drastically in order to avoid hypothyroidism (Table I). It is not certain, however, that this can be ascribed simply to a greater specific efficacy of thiourea as the hyperthyroidism came under control. Quite possibly remissions might eventually have been produced with doses of 0.07 gram daily continued from the start as well as with initial doses of 0.28 gram which were subsequently reduced. There certainly is a time as well as a dosage factor involved in the action of the drug. The experience with thiouracil in normal subjects is interesting in this respect, since it required weeks or months to induce hypothyroidism, even with large doses (18, 19).

In some of the patients at least, the hyperthyroidism was merely in remission rather than cured, since reduction in thiourea dosage or withdrawal of iodine solution was followed by a rise in serum precipitable iodine (Table I and Figure 2).

This remission was apparently produced as readily in patients with toxic adenomata as it was in those with diffuse goiter. The promptness of response varied greatly from patient to patient. The time interval necessary to produce the remission seems to be unrelated to the initial level of serum precipitable iodine, or to the severity of the hyperthyroidism.

Hypothyroidism developing during the course of prolonged medical treatment of hyperthyroidism is a regrettable complication, both because of the general reaction of the patient, and because of the possibility that exophthalmos may be favored. The difficulty of its control is enhanced by the tendency toward progressive decline of thyroid activity in many patients while receiving the same dose of thiourea. Clinically the evidences of this type of hypothyroidism are mild but distinct, consisting mainly of feelings of chilliness, drawling speech, psychomotor retardation, and sometimes excessive weight gain. The fall in basal metabolic rate often lags behind that of the serum iodine, and behind the earlier clinical symptoms, and so is not always a reliable guide. The hypothyroidism can be controlled either by administration of desiccated thyroid or by reduction in dosage of thiourea, but both these procedures entail the danger

of recurrence of the hyperthyroidism. Further studies of the optimal regime for control of the hypothyroidism are now in progress.

CONCLUSIONS

1. Thiourea and iodine medication supplement rather than interfere with one another in the treatment of hyperthyroidism. This is established (a) by the more marked remission during the first 3 or 4 months produced by the two substances in combination than that produced by thiourea alone; and (b) by the recurrence of hyperthyroidism in some patients maintained in remission by both substances when iodine alone is discontinued.

2. Preliminary iodine medication does not delay the action of thiourea, provided iodine therapy is not discontinued.

3. Thyroid activity tends to decline slowly and progressively over many months during the course of prolonged medication with thiourea. If hypothyroidism is to be avoided, this necessitates reduction of dosage of thiourea to one-fourth or even one-eighth of the amount given initially.

4. Hypothyroidism may also be corrected by oral administration of dried thyroid. The necessary dosage varies considerably from subject to subject, and lies between 0.03 and more than 0.06 gram daily.

5. Preoperative or prolonged medical therapy with thiourea and kindred drugs should in general be supplemented at all times with iodine.

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THE USE OF TWO RADIOACTIVE ISOTOPES OF IRON IN TRACER STUDIES OF ERYTHROCYTES [1]

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INTRODUCTION

There are 2 radioactive isotopes of iron suitable for use as biological tracers. One of these, Fe^{59}, has a half life of 47 days and emits gamma rays and low energy beta rays. The other, Fe^{55}, has a half life of about 5 years and emits low energy x-rays only.

This paper describes how these 2 isotopes are prepared for red blood cell tracer studies, how biological samples containing the radioactive iron are prepared for measurements, how the measurements are performed, and how the results are reported to collaborating medical research teams engaged in various physiological and clinical studies.

Fe^{59} has been in use for a number of years for metabolism and red cell studies (1 to 5) and a method of preparing samples for measurement with a thin window beta ray counter has been described by Ross (6). The longer lived isotope, Fe^{55}, on the other hand, appears not to have been used previously.

Each isotope has characteristics that make it particularly useful in certain problems; the combined use of both tracers offers solutions of problems which cannot be obtained by the employment of either isotope alone.

PREPARATION OF THE ISOTOPES

a. Nuclear bombardment. Bombardment of iron with deuterons yields both isotopes mixed. The earlier work of Hahn *et al.* (1 to 5) and Ross (6) used these mixed isotopes, but their radioactivity detection apparatus was sensitive only to the radiations from Fe^{59}. The nuclear reactions are Fe^{58} (d, p), Fe^{59} (Fe^{58}, a stable isotope, bombarded with deuterons, d, forms, through the liberation of a proton, p, Fe^{59}) and Fe^{54} (d, p) Fe^{55}. When iron is bombarded, these 2 isotopes are produced simultaneously, and cannot be isolated thereafter in a state of radioactive purity.

By deuteron bombardment of manganese, the reaction Mn^{55} (d, 2n) Fe^{55} occurs and only the long-lived radioactive iron isotope is obtained. The manganese targets are prepared from an alloy of manganese (90 per cent Mn, 8 per cent Cu, 2 per cent Si) which has suitable mechanical and thermal properties. This alloy is cast into $\frac{1}{8}$-inch slabs and machined down to $\frac{1}{32}$-inch thickness. Targets $\frac{1}{2} \times 1$ inch are cut from this material and silver soldered to the oscillating probe target described by Livingston (7). Evaporation of the target face limits the deuteron beam current to 100 μa at 14 MEV. Under these conditions our cyclotron yields of Fe^{55} have been approximately $\frac{2}{3}$ of a microcurie per microampere hour.

The shorter-lived isotope, Fe^{59}, can be produced in pure form by the bombardment of cobalt with neutrons, through the nuclear reaction Co^{59} (n, p) Fe^{59}. Deuteron bombardment of cobalt also yields Fe^{59} as a by-product of the fast neutrons always present with deuteron reactions. Because thin cobalt targets are necessary for thermal conduction, and the total amount of cobalt is small (1 to 2 grams), this is not an efficient method of producing high Fe^{59} activities. If a comparatively large piece of cobalt (about 40 grams) is soft soldered to the back of a beryllium target the larger amount of cobalt, higher permissible beam currents on Be, and much higher neutron yield

from the Be^9 (d, n) B^{10} reaction, give much better yields of Fe^{59}. Using beryllium, our yields have been approximately 0.023 microcuries per microampere hour.

b. Radiochemical preparation. Regardless of the mode of formation of the radioactive iron, the purification and preparation of material for use is essentially the same in all cases. The target material Fe, Mn, or Co is dissolved in concentrated hydrochloric acid, diluted and filtered. If manganese and cobalt of high purity are used it is necessary to add 1 to 10 mgm. of inactive Fe^{+++} as a carrier. Usually there is enough iron in the target so that such additions are unnecessary. After oxidation of the iron from Fe^{++} to Fe^{+++} with H_2O_2 the iron is precipitated as $Fe(OH)_3$ with pyridine (8).

The precipitate of $Fe(OH)_3$ is dissolved in 6 M HCl, and 10 mgm. each of Mn, Co and Zn added as carriers to aid in eliminating the radioactive isotopes of these elements from the iron. Ferric hydroxide is again precipitated with pyridine, leaving the Mn, Co and Zn in solution. This precipitation procedure is repeated 2 to 4 times until the activity in the filtrate is low.

After elimination of these 3 elements, the hydrogen sulphide group is eliminated by adding 50 mgm. of Cu^{++} as carrier for this group, and precipitating the sulphide with H_2S from a 0.1 M HCl solution. The preciptate is filtered off and discarded. The filtrate is boiled to eliminate H_2S, Fe^{++} is oxidized to Fe^{+++} with H_2O_2, and $Fe(OH)_3$ is precipitated with NH_4OH. This precipitate is dissolved in 1.5 M H_2SO_4, and 10 mgm. of indium is added (radioactive indium is formed from the cadmium in the silver solder used). Precipitation of Fe^{+++} with cupferron leaves the indium behind in the filtrate (9). The cupferron precipitate is carefully ignited, the Fe_2O_3 dissolved in concentrated HCl, diluted to 3 M, indium carrier added and the precipitation with cupferron repeated. This precipitate is ignited, dissolved in concentrated HCl, diluted, filtered (to remove unburned carbon which usually is present after igniting the cupferron precipitate) and the iron reprecipitated as $Fe(OH)_3$ with NH_4OH. This precipitate is carefully ignited in a weighed crucible and the Fe_2O_3 weighed to determine the weight of iron present.

The final step in the preparation of the iron is to synthesize it into a compound suitable for intravenous use in animals and humans. Ferric ammonium citrate, in doses described below, has been found safe for intravenous injection. After dissolving the Fe_2O_3 in concentrated HCl and diluting, $Fe(OH)_3$ is again precipitated with NH_4OH and is well washed with distilled water. The precipitate is dissolved in a solution containing 3 moles of citric acid for each mole of iron present. Warming the mixture hastens the solution of the precipitate. When the precipitate is completely dissolved the solution is brought to a pH of 7.0 with NH_4OH and filtered into a clean bottle.

This solution can be diluted with neutral distilled water to obtain any desired concentration of iron. It withstands sterilization by autoclaving if sealed in glass ampouls, but heating in open containers may result in the formation of a precipitate. Ferric ammonium citrate is photosensitive and should be kept in the dark to avoid precipitation. The compound does not inhibit the growth of common molds.

PREPARATION OF RADIOACTIVE RED CELLS

When radioactive ferric ammonium citrate is injected intravenously into animals or humans it is rapidly removed from the blood stream. A large portion of the iron is retained in the body tissues concerned with iron storage. Hahn *et al.* (10) gave ferric ammonium citrate to dogs and assayed liver and spleen for both ferritin and radioactive iron, and found a relatively high level of radioactivity in the ferritin iron. It therefore seems highly probable that the radio-iron becomes intimately mixed with all of the body iron stores present when the isotope was injected, and is hence available for hematopoietic needs.

It is generally believed that hemoglobin is "laid down" inside the developing erythrocyte. If some of the iron atoms involved in this synthesis are radioactive, a proportionate number of them will become an integral part of the new hemoglobin molecule within the newly developed cell. When the cell is released into the circulation, its presence in the blood stream can be detected as long as it remains morphologically intact. When the tagged red cell is destroyed, its

hemoglobin derived iron is very rapidly removed from the plasma, to be reused, to some extent at least, in the synthesis of new hemoglobin.

In general, each red cell contains approximately 0.1 per cent iron, or about a thousand million atoms of iron. In the radioactive donors, between 1 and 10 of these iron atoms contains a radioactive nucleus, the vast majority of the iron atoms in each red cell being ordinary stable iron.

It has been demonstrated experimentally that there is no exchange of the radioactive iron in the red cells, either with plasma *in vitro* or *in vivo*, or with saline *in vitro*. Thus the method differs in important respects from the labelling of red blood cells by absorbed carbon monoxide (11) or by adsorbed radioactive phosphorus (12). It furnishes a specific method of detecting the advent of new red cells, and to a certain extent, of following their fate in the circulation, and the disposition made of their contained iron.

The normal human erythrocyte is thought to have a life expectancy of about 100 days. Within this period it is possible to estimate the age of the oldest tagged cell in the circulation of a subject who has been given radio-iron. When these cells are removed from circulation some part of their contained radio-iron will eventually re-enter the circulation in the hemoglobin of new cells. If this "turnover" be allowed to continue for a sufficient period of time, the tagged cells will be of all ages from birth to death, or representative of a "mixed age population" of cells. The administration of the alternate radioactive isotope of iron at such a time will result in the production of new cells, the presence of which can be differentiated from the presence of "mixed age cells." Thus the behavior of young and old cells may be studied in the same subject.

Hemoglobin solutions prepared from cells obtained from donors prepared with either or both isotopes can also be used as radioactive tracer material.

The utilization of radio-iron, either when given as a ferric salt or as dissolved hemoglobin, or resulting from the postinfusion breakdown of tagged red cells, as well as the application of the "double tracer" technique to a wide variety of experimental and clinical studies will be discussed in subsequent communications (13, 14).

PREPARATION OF BLOOD SAMPLES FOR RADIOACTIVE MEASUREMENT

Both of the iron isotopes have radiations that are readily absorbed in a thick sample of material. Thus, for maximum detection efficiency all of the iron must be separated from extraneous, material, and the metal plated as a thin uniform film on a metal planchet. In this form it can be placed close to the window of the counter tube used to measure the activity. This separation is accomplished by wet ashing the sample and precipitating the iron.

The aliquot of donor cells, or the recipient's packed cells are transferred, with adequate rinsing with water, to a 500 ml. Kjeldahl flask, and concentrated H_2SO_4 added. Three to 5 ml. is adequate for donor, and 10 ml. for recipient samples. Inactive iron is added to bring the total iron content of the sample to 10 mgm. It may be assumed that 1 ml. of packed red cells contains 1 mgm. of Fe. A solution of $FeCl_3$ containing 5 mgm. Fe per ml. is convenient. The inactive iron should be added before digestion.

The acid mixture is slowly brought to a boil over a bunsen burner. Glass beads aid in reducing foaming and bumping. When the water has boiled off and the acid starts to fume, 1 ml. of 60 per cent $HClO_4$ is added, slowly, and this is repeated a few times until digestion is complete. Not more than 4 ml. of $HClO_4$ should be added to the original 10 ml. of acid, and it should *never* be added if the H_2SO_4 has boiled dry, as an explosive compound is formed. As digestion proceeds, the boiling becomes less violent, and more heat can be applied. When digestion is complete the liquid will be a straw yellow color when hot. On cooling it becomes colorless and a chalk white precipitate settles out. All organic matter must be completely oxidized or the subsequent precipitation of iron may be incomplete.

The acid residue is then transferred to a 100 ml. centrifuge tube with 1 or 2 washings with 0.1 N H_2SO_4. The iron is then precipitated by the addition of concentrated NH_4OH to slightly beyond the neutral point. A fine brown precipitate which becomes flocculent appears at the end point. A mechanical stirrer increases the speed with which ammonia can be added. The tube is centrifuged at 1200 r.p.m. for 10 minutes and the

supernatant fluid removed by suction. Since the precipitate is fine, it is well to use a filter paper over the end of the suction tube. The iron is then ready for electrolytic deposition on the copper counting planchets.

Iron can be plated quantitatively from an ammonium oxalate-oxalic acid solution (15). The technique of electroplating described herein is a modification of the method of Ross (6). The $Fe(OH)_3$, in the centrifuge tube in which it was precipitated, is dissolved in 0.5 ml. of 3 M H_2SO_4. This solution is transferred to the plating cells (Figure 1), using a saturated ammonium oxalate solution. The final volume is about 25 ml., and the pH of the solution is 4 to 5.

Electrolysis is carried out at 0.9 amp. with a voltage drop across the cell of about 6 to 8 volts. Six cells are run in series to avoid unnecessary power dissipation when 110 v. DC is used as a source of current. A small rheostat and an ammeter serve as a control and a monitor for the groups. Provision is made for shunting each cell through a 6 to 8 volt light bulb of the proper resistance so that any number of the 6 cells can be used at once. At the end of an hour the pH of the solution is tested. If it is greater than pH 7,

a few drops of 3 M H_2SO_4 are added to bring it down to about pH 6, and electrolysis is continued for a total of 1.5 hours. A 0.5 ml. sample is withdrawn and tested for completeness of deposition of the iron. If this sample contains more than 0.0006 mgm. of iron, the pH is readjusted to between 6 and 7, and the plating continued for another half hour.

In testing for iron, advantage is taken of the intense red color developed when ferrous iron is treated with a, a' dipyridyl in an acid solution. The sample to be tested is made acid with a drop of 3 M H_2SO_4 and 1 drop each of 1 per cent a, a' dipyridyl in 0.5 M HCl and 10 per cent $NaHSO_3$ is added. Gentle warming develops the color in about 2 minutes. The amount of iron in solution is determined by comparing the color developed with permanent color standards in a simple color comparator. These standards are made by visual matching of cobalt chloride solutions with the color developed in iron solutions of known concentrations. When properly matched, the standards are sealed into small 100 mm. test tubes and permanently mounted in the comparator.

After the iron has been completely plated from the solution (less than 0.0012 mgm. per ml. remaining) the planchet is removed and dried with an air blast. It is then coated with a film of light machine oil diluted with benzene 1:100.

The sample is now on a copper planchet 1 inch in diameter and 0.02 inch thick (a form readily available commercially). The iron film forms a circle $7/8$ inch in diameter and contains 10 mgm. of iron or 2.6 mgm. Fe per cm². It is now ready for measuring.

COUNTERS WHICH DIFFERENTIATE BETWEEN THE TWO RADIOACTIVE ISOTOPES OF IRON ON A BASIS OF THEIR NUCLEAR SPECTRA

Deutsch *et al* (16) in cooperation with the authors made a study of the radiations emitted by Fe^{59} (47 day half-life). They found that this isotope disintegrates by either of 2 similar processes, each with about the same probability of occurrence. In one case a beta ray, or energetic electron, is emitted with 0.46 million electron volts (MEV) maximum energy, followed by a gamma ray of 1.10 MEV. In the alternate method of disintegration the beta ray has a maximum energy
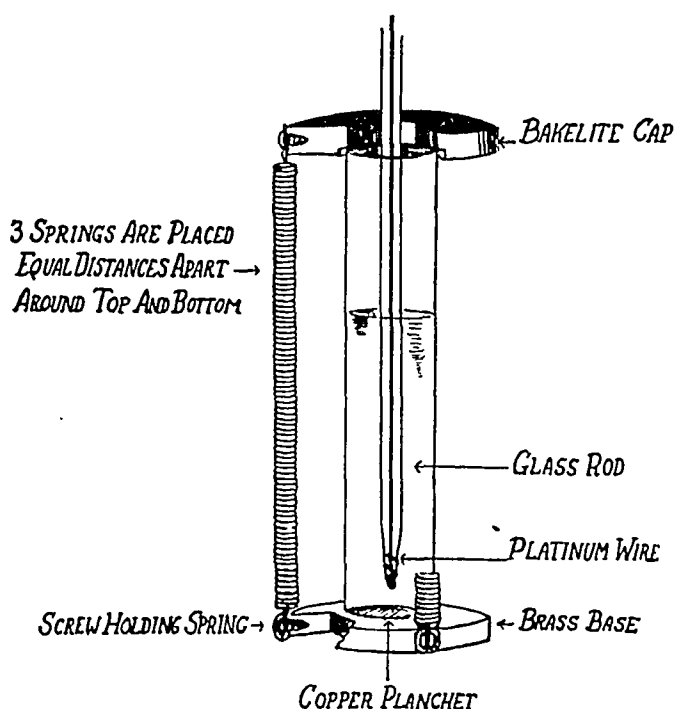

FIG. 1. SEMI-DIAGRAMMATIC VIEW OF THE APPARATUS FOR ELECTROPLATING IRON ON PLANCHETS FROM SOLUTION, ASSEMBLED

Each unit has individual motor drive.

of 0.26 MEV, and is followed by a gamma ray of 1.30 MEV. In either case, the beta rays emitted have an average energy of only about ⅓ that of the maximum. The average energy of all the beta rays is then only about 0.12 MEV.

Beta rays are absorbed to about the same extent by equal masses of any material, the fraction transmitted through any in particular being dependent upon the initial energy of the individual beta rays. The counters used for measuring Fe^{59} are filled with helium to about, 70 cm. Hg pressure and they have a mica window approximately 10 microns thick. The effect of the counter window thickness on the transmission of these beta rays is shown in Figure 2. This emphasizes the advantage of using thin counter windows when measuring Fe^{59} (6).

Fe^{55} (5 year half-life) disintegrates into Mn^{55},

a stable isotope, when the nucleus captures an electron from one of the inner orbits of the atom. . This process, "orbital electron capture," leaves a vacancy in one of the inner electron shells which is subsequently filled by an electron from an outer shell. As a result, an x-ray characteristic of the Mn^{55} atom may be emitted. The most abundant and most energetic x-ray emitted has an energy of 5.9 kilovolts (Ka_1 line of Mn). One of these x-rays leaves the atom in about 24 per cent of the disintegration.[3] Any radiation other than this one x-ray that might be expected in connection with

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[3] This x-ray can be emitted only if a K shell electron is captured, about 80 per cent of the cases, and in only 30 per cent of these K capture processes is an x-ray emitted from the atom. In the other 76 per cent of the cases, low energy electrons (Auger electrons) and L and M x-rays are liberated but are not detected.
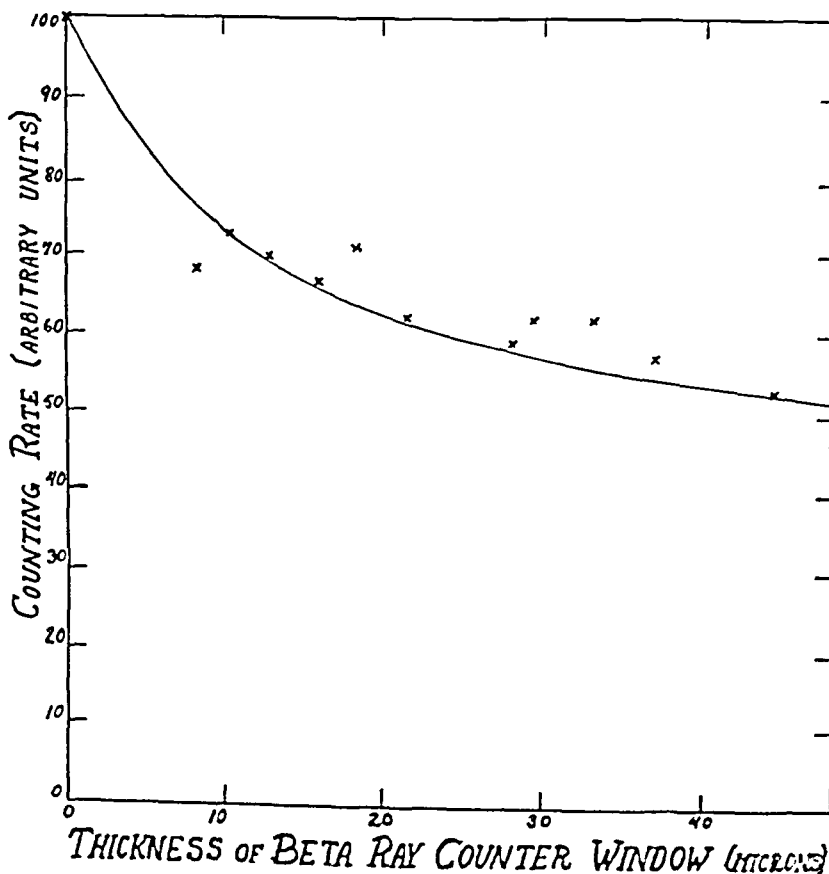
HELIUM
FILLED
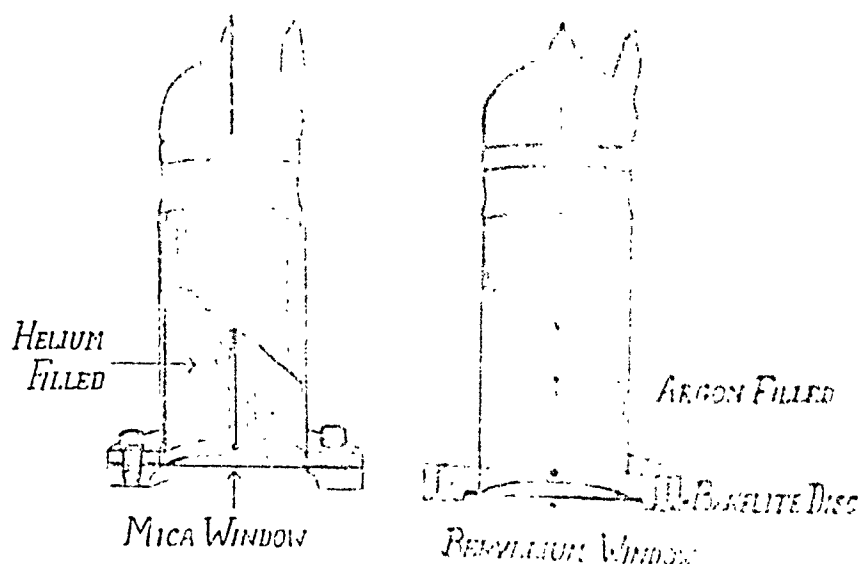
ARGON FILLED

BAKELITE DISC

MICA WINDOW

BERYLLIUM WINDOW

FIG. 3. THE HELIUM FILLED COUNTER USED TO MEASURE BETA RAYS
FROM Fe⁵⁹ IS SHOWN ON THE LEFT

The argon filled counter for detection of x-rays from Fe⁵⁵ is shown on
the right. The counters are differential since the x-rays are not absorbed
in the helium, and beta rays are absorbed in the beryllium window.

this disintegration (e.g.: gamma rays, conversion electrons, etc.) has not been observed with our present detection techniques. Table I lists the mass absorption coefficients and the thicknesses of various materials necessary to attenuate this 0.0059 MEV x-ray to half value. In every case the absorption is exponential. The striking thing that will be noted, besides the relatively small amount of material necessary to absorb the radiation, is the side difference in the absorbing power for the same mass of different materials (proportional to the cube of the atomic number).

Thus a 10 micron mica window would transmit 57 per cent of the x-radiation, and a beryllium window 0.76 mm. thick would transmit 55 per cent of the x-radiation. Such a beryllium window would transmit less than 5 per cent of the beta rays from the 47 day iron isotope, while a 10 micron mica window will transmit 75 per cent of the beta rays.

Any agent that will produce ionization in the "counting volume" of a Geiger Counter will cause it to "count." In order for the x-ray to be counted it must be absorbed in the gas of the counter, or near enough to the surface of the walls so that

electrons formed by the x-ray will get to the counting volume. In order for the Kα x-ray of Mn to cause a count it must be absorbed either in the gas of the counter, or in the walls of the counter at a depth not greater than would be covered by 14 mgm. per cm².

Thus it is found that a counter with a beryllium window thick enough to stop 47 day iron beta rays and filled with argon gas at 60 cm. Hg pressure detects about 30 times as many x-rays as the 10 micron mica window counter filled with helium at 70 cm. Hg pressure.

These two types of counters (Figure 3) are useful in double tracer experiments, since each will detect the presence of one isotope and is almost completely insensitive to the other.

Ross (6) has already pointed out that with the 47 day isotope the fraction of beta rays absorbed within a source of the present geometry (2.6 mgm. per cm²) is negligible, but that an appreciable fraction of the beta rays are absorbed as the source gets thicker (approximately 10 per cent are absorbed with a 5.2 mgm. per cm² source).

Figure 4 shows the effect on counting rate of plating increasing amounts of inactive iron with

sample containing the same amount of Fe^{55}. The curve is proportional to $\dfrac{1 - e - Nt}{Nt}$ where N is the mass absorption coefficient of K Mn x-rays, e is the base of the natural logarithm, and t is the thickness of the iron in grams per cm^2. The points represent measured activities using increasing amounts of stable iron. There is approximately 1 per cent self absorption per mgm. of sample under these conditions. One readily sees from this figure that it is even more important to keep constant sample weight in the case of Fe^{55} than with Fe^{59}.

SAMPLE MEASUREMENTS

The copper planchets on which the iron has been plated are placed on a turntable which automaticaly places one sample after the other under the counters which are to measure the activities of the iron (Figure 5). Each sample is measured for a predetermined time interval (usually 15 or 30 minutes). As a new sample comes under the counter its counts begin to register on a counting-rate-meter. The average counting rate is registered in ink on the tape of a recording milliammeter. If both iron isotopes are used in the experiment the records from a beta ray counter
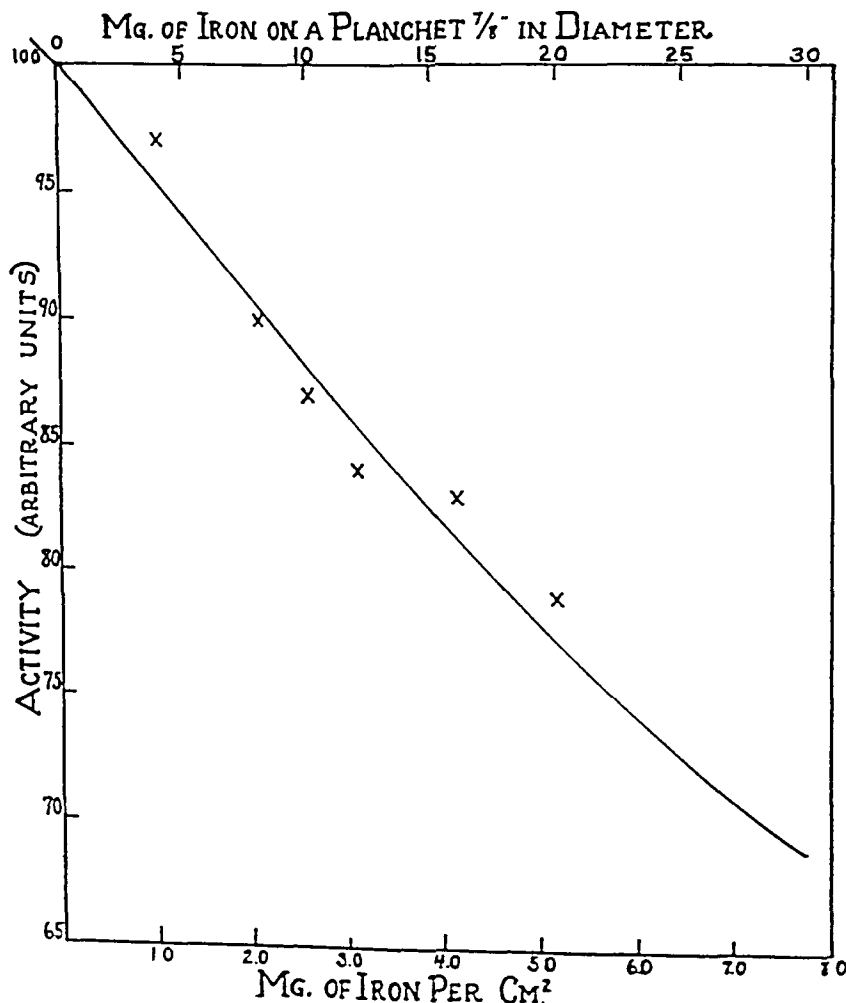

FIG. 4. A CONSTANT AMOUNT OF Fe^{55} AND VARYING AMOUNTS OF INACTIVE IRON WERE PLATED TOGETHER

The points are proportional to the measured activities and the solid curve is the predicted activity if all detected radiation is the K x-rays associated with the isotope. Both the curve and the experimental points are normalized to 100 at zero thickness of iron.
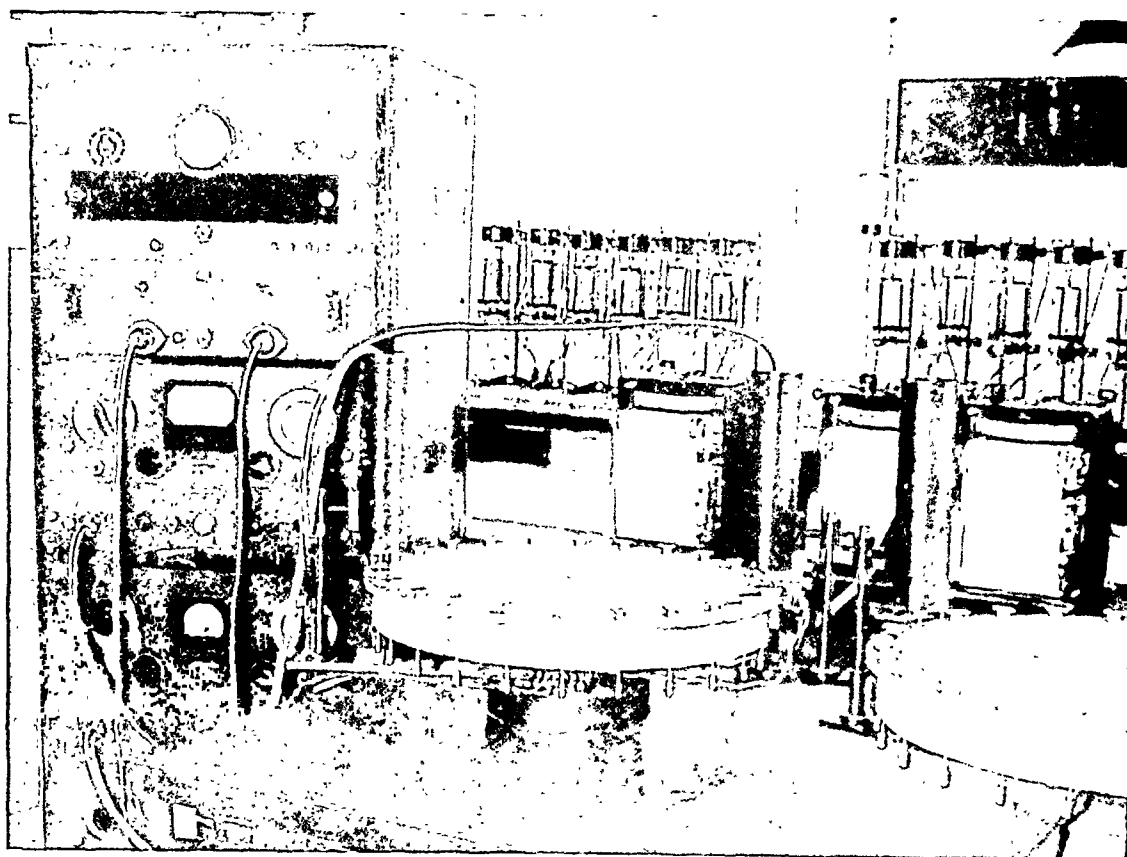
FIG. 5. APPARATUS FOR MEASURING THE RADIOACTIVITY OF Fe^{55} AND Fe^{59} SAMPLES

Two batteries of electroplaters appear in the background. A beta ray counter is mounted on the left and an x-ray counter on the right side of the automatic sample changer, in center foreground. Planchets are secured to the plungers. The turntable rotates through 15° every 15 minutes; the counted planchet drops, and the next sample is moved up close to the counter window. Counting rate meters and calibrating apparatus appear at the left, and the pen and ink recorders in the middle background.

and an x-ray counter are obtained separately and simultaneously and on separate charts, simply by putting both types of counters over the same turntable.

To insure accuracy in measurement, the apparatus is checked regularly for changes in calibration, background, or counter sensitivity.

METHOD OF REPORTING RESULTS

All activity measurements are reported in terms of "unit activity" or Ua. We obtain this quantity as follows: the background counting rate is subtracted from each sample reading to give net counts per minute. Net counts per minute in a sample are divided by the total volume of red cells in the sample and the net counts per minute in the radioactive standard used. The radioactive standard is an aliquot of the radioactive material originally administered to the subjects used in the

experiment. Thus Ua is independent of radioactive decay and counter sensitivity. Ua is proportional to the relative concentration of radioactive iron found in the various samples measured. From the known weight of iron in the aliquot used as the standard, one can readily calculate the number of micrograms of tagged iron corresponding to a Ua of one.

Suppose for example 1 ampul of 5 year iron contained 1 million counts per minute as detected at a particular time on an x-ray counter, and that there was 1 mgm. of iron in the ampul. Three thousand counts per minute would be a convenient counting rate, so a standard would be prepared containing 3 micrograms of radioactive iron and 10 mgm. of inactive iron. A sample of red cells having 1500 counts per minute per ml. would have a Ua of 0.50. Assuming all this activity was from the same iron target as the stand-

ard, the blood would contain 1.5 micrograms of the tagged iron from the target per ml. of red cells. It is worth while emphasizing at this point, however, that standards of 5 year iron that are used continuously for a period of weeks will not have the same counting rate at the end of this time as samples freshly plated. This is due to the absorption of the radiation in a thin oxide film that forms over the planchet surface.

RADIATION RECEIVED BY A DONOR WITH RADIOACTIVE BLOOD

There is one more important consideration in connection with the physical aspect of this study, *i.e.* radiation that will be received by the donor to whom the radioactive isotopes are given.

The activity a donor's blood should have varies with the type of experiment being performed. Let us take as an example a case where about 3000 counts per minute per ml. of donor red cells are necessary in order to obtain sufficient activity in the recipient cells with either isotope.

The present beta ray counters detect approximately 25 per cent of the disintegrations of Fe^{59}, and the x-ray counters detect approximately 3 per cent of the disintegrations of Fe^{55}.

We can assume that essentially all of the gamma ray radiation of Fe^{59} is absorbed outside the blood stream, and that the large majority of it is not stopped inside the body at all. The beta rays of Fe^{59} have a maximum energy of 0.46 MEV and an average energy of 0.12 MEV. Hence (17) their maximum range is about 1.5 nim., and their average range is about 0.15 mm. of blood or surrounding tissue. The mass absorption coefficients (Table I) for the x-rays of Fe^{55} (5.9 kv.) show

that these rays are also absorbed in a comparably short distance in tissue. We can obtain a sufficiently accurate numerical estimate of the maximum radiation dose by assuming that all the beta rays and x-rays are completely absorbed within the blood stream. The actual radiation dose per gram will be somewhat less than this estimate, because of the portion of the rays absorbed by tissues, especially those surrounding the smaller blood channels.

In x-ray practice, tissue radiation doses are expressed in roentgen units, and it is convenient to express the radiation doses due to radioactive isotopes in these same units. One roentgen is that quantity of radiation which produces 1 electrostatic unit (esu) of ions, of both signs, in 1 ml. (0.001293 gram) of air at 0° C. and 760 mm. Hg pressure. Because each ion carries a charge of 4.80×15^{-10} esu, 1 roentgen amounts to $1/(4.80 \times 10^{-10} \times 0.001293) = 1.61 \times 10^{12}$ ion pairs per gram of air. The average amount of energy required to produce 1 ion pair in air is (18) 32.5 electron volts, or $32.5 \times 1.60 \times 10^{-12} = 52 \times 10^{-12}$ ergs. Because tissue is composed of elements having a low atomic weight similar to air, the energy required to form an ion pair in tissue may be taken as the same as the energy required to form an ion pair in air. Then 1 roentgen represents an energy expenditure in each gram of tissue of $32.5 \times 1.61 \times 10^{12} = 52 \times 10^{12}$ electron volts, or of $52 \times 10^{-12} \times 1.61 \times 10^{12} = 84$ ergs.

One ml. of donor blood, at a hematocrit of 40 per cent, will then contain $3000 \times 0.4 = 1200$ c.p.m. When the efficiency of the counters is considered, these counting rates correspond to the disintegration of $1200/0.25 = 4800$ atoms of Fe^{59}, or of $1200/0.03 = 40,000$ atoms of Fe^{55}.

The maximum energy delivered per gram (0.95 ml.) of blood by Fe^{59} is thus $4800 \times 0.95 \times 0.12 \times 10^{6} = 5.5 \times 10^{5}$ electron volts per minute or 5.5×10^{12} electron volts per week, or $5.5 \times 10^{12}/52 \times 10^{12} = 0.11$ roentgen per week.

Similarly, the maximum energy delivered per gram of blood by Fe^{55} is $40,000 \times 0.95 \times 5.9 \times 10^{3} = 2.2 \times 10^{8}$ electron volts per minute or 2.2×10^{12} electron volts per week, or $2.2 \times 10^{12}/52 \times 10^{12} = 0.04$ roentgen per week.

Thus the radiation dose from either isotope in a donor may be taken as 0.1 roentgen per week in

TABLE I

Material	Mass absorption coefficient in cm³ per gram	Thickness in cm. necessary to reduce intensity to half
He (gas at S.T.P.)	0.5	7800.
Be	4.3	0.087
Carbon (graphite)	11.	0.028
Oxygen (gas at S.T.P.)	28.	17.5
Mica*	210.	0.0012
Aluminum	130.	0.0019
Iron	100.	0.00087
Argon (gas at S.T.P.)	286.	1.35
Gold	480.	0.000076

* $K_2O \cdot 3 Al_2O_3 \cdot 6 SiO_2 \cdot 2H_2O$.

the blood stream only, and a negligible dose in the rest of the body tissues. Alternately, if the radiation is assumed distributed uniformly through all the body tissues, then the whole body radiation dose is about 0.007 roentgen per week for Fe^{59} donors, and 0.003 for Fe^{55} donors. In recipients the radiation doses are much smaller than in the donors, usually by a factor of 50.

The tolerance dose for continuous whole-body exposure is 0.1 roentgen per day, or about 1 roentgen per week to all the tissues of the body (19).

It is evident that no radiation effects are to be expected, and none have been observed. We have built up a total of 48 human donors. Three received the isotopes Fe^{55} and Fe^{59} mixed (produced by bombardment of an iron target), 6 received Fe^{59}, and 38 received Fe^{55}, 3 of the latter also receiving subsequent doses of Fe^{59}. The calculated dosages in these donors have ranged from 0.05 to 0.2 roentgens per week. Three of these subjects have been observed over a period of 4 years, 8 for from 12 to 19 months, and 36 for from 2 to 10 months.

Red cells from these donors have been transfused into 160 human recipients in amounts ranging from 50 to 250 ml. These subjects have been under observation for from 2 months to 4 years.

All of these subjects were normal young adult males, with active daily routines. In no instance has any change in blood picture occurred, as evidenced by changes in hematocrit or hemoglobin levels (other than could be accounted for by bleeding), or change in leucocyte count. Regeneration of red cells following bleeding has apparently been normal, and several donors have been bled more than once. Three subjects have married subsequent to receiving radio-iron, and have begotten normal children.

In addition, radio-iron, in dosages about equivalent to that received by recipients, has been given to 65 patients on the wards of the Peter Bent Brigham Hospital, without observed radiation effects. Many of these patients had anemias, and yet the course of recovery from hemorrhage appeared to be unaffected by the radiation.

SUMMARY

1. Two radioactive isotopes of iron, Fe^{59} (47 day half life) and Fe^{55} (5 year half life), are pro-
duced separately and obtained in a state of radioactive purity. These isotopes are prepared in the form of ferric ammonium citrate for intravenous injection into human subjects. The radiation doses in donors and recipients are well below accepted tolerance levels.

2. Blood and tissue samples are prepared for radioactive measurement by H_2SO_4 — $HClO_4$ digestion and ammoniacal precipitation. The iron in the sample is electroplated from an ammonium oxalate-oxalic acid solution onto a copper disk.

3. Routine measurements are reported in terms of "unit activity" which is a ratio of sample to a standard aliquot of the original activity. Corrections for decay during an experiment are thus eliminated.

4. Geiger-Müller counters are described, which discriminate between the 2 isotopes of iron when both are present in the same biological sample, thus permitting double-tracer experiments.

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THE MEASUREMENT OF THE CIRCULATING RED CELL VOLUME BY MEANS OF TWO RADIOACTIVE ISOTOPES OF IRON [1]

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Measurement of blood volume, both of its plasma and red cell portion, is a useful tool in clinical and experimental investigation. It provides basic data for the assay of the total quantity of individual blood components, as well as for the evaluation of observed hemodynamic changes in acute or chronic disturbances of the circulation. Blood volume determinations have proved of value in the study of congestive heart failure, the anemias, thyroid disorders, renal disease, and pregnancy; and they have been particularly useful during the recent war years in the study of hemorrhage, burns and shock, and in the development of blood substitutes and blood cell preservatives.

It seems reasonable to state that the dye method (1, 2) measures the circulating plasma volume with a high degree of accuracy both in normal and pathologic states, and in many clinical and experimentally induced abnormal circulatory conditions. This view appears to be shared by investigators who have had firsthand experience with the technique (3, 4).

Perhaps the best experimental evidence that the method accurately measures changes in plasma volume was obtained in a series of experiments in which the increase in plasma volume following the intravenous injection of concentrated (25 per cent) human serum albumin was measured (5). The osmotic equivalent of 1 gram of this protein was found by Scatchard et al (6) to be 18 ml. of H_2O; the average increase in plasma volume in 11 male human subjects was 17.4 ml. of H_2O (5) per gram of albumin.

Total blood volume and red cell volume can be calculated from the plasma volume and the venous, auricular or arterial hematocrit. This indirect measurement is based on the assumption that the ratio of cells to plasma is a constant throughout the entire vascular tree. It has long been suspected that this is not true.

Smith et al (7) found that the red cell volume as determined by the Welcker method (exsanguination) and the carbon monoxide method was consistently lower than when calculated from the hematocrit and plasma volume measured by the dye method of Keith, Rowntree and Geraghty (8). Since the first 2 methods were independent of the hematocrit, they concluded that the cell to plasma ratio was different in large and small vessels.

Fåhraeus in 1929 (9) reported experiments in which normal blood was allowed to stream through glass capillary tubes ranging from 1.1 to 0.050 mm. in diameter. The ratio of cells to plasma remained fairly constant, averaging about 40 per cent, until the diameter of the tube was less than 0.1 mm., but as the diameter was further decreased, the proportion of erythrocytes fell rapidly, being only 28 per cent in tubes of 0.050 mm. (50 microns). This size of tube is comparable to the larger capillaries. An even lower hematocrit might be expected to occur in blood flowing through smaller capillaries.

The problem was studied by Stead and associates in normal humans (10) and in splenectomized dogs (11) in 1940–41. Plasma and red cell volumes were determined by the dye method prior to a single large hemorrhage. These observations were repeated at intervals during the next 4 to 6 days. The expected cell volume at the time of repeated volume determinations was calculated by subtracting the quantity of red cells removed

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by hemorrhage, and by subsequent sampling from the original cell volume. As the hematocrit of the venous blood fell due to hemodilution the observed cell volume became progressively less than the expected cell volume, even though the observations were made during a period when some regeneration of red cells may have been taking place. If, to the lowest observed cell volume the total quantity of red cells removed was added, the sum was always less than the observed pre-hemorrhage volume.

These studies supported the view that the red cell volume, as measured by the dye-plasma-hematocrit technique, was always higher than the true red cell volume.

The use of a diffusible gas, CO, having a high affinity for human hemoglobin, was suggested as a means of tagging red cells by Chang and Harrop (12). Following inhalation of CO, a certain proportion would be absorbed into and remain within the red cells for a considerable period. The quantity of gas absorbed by the individual cell would then vary with the partial pressure of CO in the pulmonary alveoli, but not with the ratio of cells to plasma in the blood stream. Hence the measurement, obtained from CO analysis of whole blood samples was, in theory, a measure of total circulating blood volume. The plasma volume could then be calculated from the large vessel hematocrit.

Hopper et al (13) recently reported on simultaneous measurements of blood volume in man and dog by means of Evans blue and CO. No consistent discrepancy in the results of the 2 methods was observed in normal subjects. The ratio of dye to CO determined total blood volume ranged from 0.91 to 1.16 in humans, and from 0.84 to 1.16 in normal and splenectomized dogs. The numerical average of these ratios was very close to unity. A more variable relationship was found in abnormal subjects. Dye volumes were considerably higher than CO volumes in dogs in experimental traumatic shock, the discrepancy tending to increase as the hematocrit rose with hemoconcentration (14).

Just as the dye plasma volume technique is based on controlled studies of the mechanism of the removal of dye from the blood stream, the CO method must be carried out with some regard to the fate of the gas in the body. At the present time this knowledge is far from complete.

In 1940, Hahn and Hevesy (15) labelled the phosphatides, and acid-soluble organic phosphorus compounds of rabbits with radioactive phosphorus (P^{32}). Some of these tagged compounds were incorporated in newly developing red cells, or entered them by interchange between circulating cells and plasma. These tagged cells were injected intravenously into other rabbits, and blood samples were taken at intervals thereafter. The phosphatides of both donor and recipient cells were extracted, and the contained phosphorus was precipitated and measured for radioactivity with a Geiger counter. Total blood volume was computed from the ratio of the radioactivity, expressed as units per ml. of whole blood, of donor and recipient samples. It was found that the recipient's red corpuscle content of P^{32} rapidly diminished after the injection of tagged cells, a fact which imposes several limitations on the use of the technique. The basic measurement was the radioactivity of the corpuscles; the assumption again being that the cell to plasma ratio was a constant throughout the vascular system.

All of these studies pointed to the desirability of a simple, accurate and dependable method of determining circulating red cell volume which could be used simultaneously with plasma volume determinations and other circulatory studies.

Hahn et al, in 1942, described a method of measuring red cell volume by means of a radioactive isotope of iron (16). They found that radioactive iron administered to dogs becomes incorporated in the hemoglobin of new erythrocytes, and that these cells do not release this radio-iron as long as they remain intact. When such tagged cells are given to another dog, the degree of dilution thereof in the non-radioactive cells, determined by radioactivity analyses of samples of recipient's cells, gives a measurement of total circulating red cell volume. This value is independent of variations of the cell-plasma ratio throughout the vascular tree. Since the recipient's cell radioactivity levels do not vary to any extent in from 1 to 3 days after the infusion of tagged cells, it is evident that all of the transfused cells are well retained and become completely mixed with all the cells in the circulation.

These workers simultaneously determined the dye-plasma-hematocrit cell volume and radio-iron cell volume in 13 dogs. The cell volume calculated from the plasma volume was higher than the radio-iron cell volume in all cases, the ratio of the latter to the former value ranging from 0.61 to 0.92, with an average of 0.71. Their report constitutes the first study of circulating red cell volume by a reliable specific method. Their results confirm previously expressed theoretical and experimental reasons for ciriticism of the dye-plasma-hematocrit cell volume determination.

The methods described herein are elaborations of the original technique of these workers for the purpose of applying it to a wide variety of experimental and clinical studies in animals and humans.

The measurement of red cell volume by means of erythrocytes tagged with radioactive iron is based on two assumptions, (1) that none of the radioactive atoms of iron pass out through the membrane of the intact erythrocytes, and (2) that all of the transfused tagged cells become thoroughly mixed with all of the recipient's cells; i.e., the ratio of radioactive to non-radioactive cells becomes, and remains a constant throughout the entire vascular tree, and hence is independent of differences in hematocrit levels in large and small vessels.

The validity of the first assumption has been demonstrated by *in vitro* experiments. Radio-iron dog cells were carefully washed in isotonic saline and added to whole heparinized blood from a dog which had never received radio-iron, and thoroughly mixed. The mixture was then centrifuged. No hemolysis occurred. There was no detectable radioactivity in aliquots of the supernatant plasma. Repeated washing of the cells with isotonic saline also failed to show any detectable radioactivity in the washings.

The validity of the second assumption has been established both indirectly by *in vitro,* and directly by *in vivo* experiments in dogs and humans. Compatible radioactive human erythrocytes were added to heparinized whole blood from a normal human. The experiment simulated the repeated determination of circulating cell volume before and after a hemorrhage *in vivo*. The data from a typical experiment, given in Table I, show that recovery was complete within ± 3 per cent.

Unanesthetized animals, 1 normal and 1 splenec-

TABLE I
Recovery of radioactive cells added to non-radioactive whole human blood

Procedure	Unit activities[*]		Per cent recovery
	Calculated	Found	
Radioactive cells added	0.647	0.672	103.5
Non-radioactive whole blood added	0.368	0.359	97.5
Whole blood removed and radioactive cells again added	1.480	1.500	98.7

[*] Unit activity = $\dfrac{\text{cpm per ml. of cells}}{\text{cmp of standard Fe}}$

tomized, were bled amounts insufficient to produce any appreciable fall in mean arterial pressure, and radio-iron cell volumes were determined before and after hemorrhage. The final cell volume plus the known quantity of cells withdrawn was equal to the prehemorrhage cell volume within ± 3.0 per cent (Table II).

In similar experiments in 2 moderately anemic patients the increase in cell volume as measured by radio-iron before and after transfusions of fresh compatible blood was equal to the known

TABLE II
Measurement of total circulating red cell volume before and after hemorrhage in unanesthetized dogs

	Red cell volume	Error
Dog No. 4—Splenectomized		
	ml.	*per cent*
Before bleeding	527	
Loss of red cells (direct measurement)	190	
Calculated volume after bleeding	382	
Volume measured 24 hours after bleeding	373	−2.3
Dog No. 6—Normal		
	ml.	
Before bleeding	645	
Loss of red cells (direct measurement)	135	
Calculated volume after bleeding	510	
Volume measured 3 days after bleeding	520	+2.0
Dog No. 7—Normal		
	ml.	
Before bleeding	625	
Loss of red cells (direct measurement)	350	
Calculated volume after bleeding	275	
Volume 6 days after bleedings	260	−5.4
Infusion of Beef Albumin	230	
Calculated volume after infusion	230	
Volume measured after infusion	220	−4.3

amount of cells infused within ± 2 per cent (Table III).

The fact that the addition or subtraction of known amounts of red cells to the circulation is accurately measured in these experiments indicates that the tagged red cells have become intimately

TABLE III

Measurement of total circulating red cell volume before and after transfusion in patients with anemia

Procedure	Red cell volume	Error
Patient O.O. Aged 56. Anemia due to blood loss		
	ml.	*per cent*
Before transfusion	515	
Net cells given (direct measurement)	400	
Calculated volume after transfusion	915	
Measured volume 20 hours after transfusion	905	−1.1
Patient C.D. Aged 23. Idiopathic hypochromic anemia		
	ml.	
Before transfusion	820	
Net cells given (direct measurement)	800	
Calculated volume after transfusion	1620	
Measured volume 21 hours after transfusion	1590	−1.9

mixed with the other red cells in the blood vessels. Positive evidence that this is the case lies in the fact that the ratio of radioactive to total cells does not change after the removal of a quantity of whole blood (Figure 4).

Preparation of radioactive red cell donors.

Human donors whose cells were of blood group-O (so-called universal donors) have been used routinely in these studies, since their cells are not agglutinated by the serum of any of the 4 blood groups. We have selected Rh + donors, since the majority of humans are Rh +. It is hardly necessary to point out the hazards of giving Rh + cells to Rh − recipients; but Rh − donors can be prepared. It is advisable that donor serum have low anti-A and anti-B titers.

The preparation of the two isotopes, Fe^{π} and Fe^{π}, and their synthesis into ferric ammonium citrate and preparation for intravenous use has been described in a previous communication (17). No effects attributable to radiation from either isotope have been observed following intravenous injection of neutral aqueous solutions of ferric ammonium citrate in the dosage used: 1 mgm. or less per injection (17).

The efficiency of utilization, in normal humans, of both isotopes of radio-iron, when given intravenously in small doses (1 mgm.), is striking. Radioactive cells are detectable in the blood stream within 24 hours after administration, the maximum level being attained in about 21 days. Thereafter the cell radioactivity level fluctuates little, being affected chiefly by the decay of the isotopes, the withdrawal of red cells, and the natural excretion of body iron. Bleeding prior to radio-iron administration has been found to have little or no effect on utilization, even in individuals in normal iron balance, probably because the amounts given represent less than 1 per cent of total iron stores.

The utilization of radioactive iron following a single intravenous injection has been carefully observed in 3 normal males. The percentage of the administered radio-iron utilized was calculated from the known quantity of radioactivity given, the subject's red cell volume (estimated from plasma volume and hematocrit corrected by a factor of 0.85), and the radioactivity of red cell samples taken daily. The maximum utilization was about 80 per cent.

The data obtained are shown in Figure 1, in which the uptake is shown as a percentage of the total utilization during the period of observation. It will be noted that the experimental data conform closely to an exponential curve beginning 24 hours after injection, having a half period value of approximately 3½ days. Since the radioactivity of blood samples derives only from new cells in active circulation, this curve is also an index of the rate of withdrawal of radio-iron from the body iron stores.

The quantity of radio-iron necessary to administer to a donor depends upon the specific radioactivity (counts per minute per mgm. of iron), of the individual lot of iron, and the radioactivity level per ml. of donor's cells required for the intended experiments. Each lot of ferric ammonium citrate varies in specific activity, and also, as finally prepared, in the concentration of the solution. It has been our practice to dilute each lot so that each 10 ml. ampul contained approximately 1 mgm. of Fe^{+++}, and this has been used as a standard dose. Specific activities of Fe^{π} have ranged from $1 \times 10^{\text{o}}$ to $6 \times 10^{\text{o}}$ c.p.m. per mgm. Fe per ampul, and of Fe^{π} from $1 \times 10^{\text{o}}$ to 2×10^{7}. Thus the preparation of donors can be accomplished in a short time with a few injections.

Since the requirements of each type of experiment vary, no hard and fast set of rules for all procedures can be laid down.

Based on observations of over 40 normal human donors, several of whom have received both isotopes, it seems safe to say that at least 60 per cent of the iron injected will be utilized whether given in single or multiple injections. Figure 2 is a nomogram showing the approximate radioactivity level of donors' red cells resulting from a total dosage of from $1 \times 10^{\text{o}}$ to 1×10^{7} c.p.m., in normal individuals having red cell volumes ranging from 1000 ml. to 3000 ml. Variations in individual uptake will occur, but the nomogram has proved useful as a first approximation in the preparation of donors.

With present counters used by us, sufficiently accurate measurements can be made when a given red cell sample contains a total of about 250 c.p.m. above background. Since about 5 ml. of cells are taken for each sample, the

radioactivity of the donor cells should be such that, after mixing, the recipient's blood contains about 50 c.p.m. per ml. of cells. Assuming the cell volume of a large normal adult male is 2500 ml., then the transfused tagged cells should contain a total of 125,000 c.p.m. It has been found practical to infuse about 100 ml. of donor's whole blood, containing 40 to 50 ml. of red cells. It follows that an adequate donor level may range from 2500 to 3000 c.p.m. per ml. of cells. The quantity of cells given is equal to about 2 per cent of the normal red cell volume, well within the limits of error of the technique. Thus the recipient's cell volume is not materially changed by the infusion.

Some degree of latitude obtains, but in general, the quantity of donor cells required for a single volume determination can be closely estimated. A nomogram for the purpose is given in Figure 3. This shows the minimum quantity of donor's cells, with radioactivity levels ranging from 500 to 5000 c.p.m. per ml. that must be given to individuals having red cell volumes as high as 3000 ml. to obtain a level of at least 50 c.p.m. per ml. of recipient's cells.

A brief study of these nomograms indicates that for single volumes in normal adult humans, an infusion of from 70 to 100 ml. of whole donor blood (30 to 50 ml. of red cells) having an activity of about 2500 c.p.m. per ml. will give satisfactory recipient cell radioactivity levels in individuals having red cell volumes of from 1500 to 2500 ml.

These radiation levels are quite safe for donors and we have had donors with twice these levels who have shown no untoward effects. Higher levels are unnecessary for routine cell volume measurements. Donors have been used repeatedly over periods for as long as 3 years. Since the decay rate of Fe^{59} is 10 per cent per week, occasional booster doses may be necessary and can be given without increasing radiation risks. No booster doses are needed when the 5 year isotope, Fe^{55}, is used.

The determination of red cell volume.

The techniques described below have been developed in our laboratories for the measurement of cell volume in humans and dogs. The underlying principles are, however, applicable to a variety of experimental procedures each imposing a diversity of limiting factors. The techniques are identical for blood samples containing either one or both isotopes.
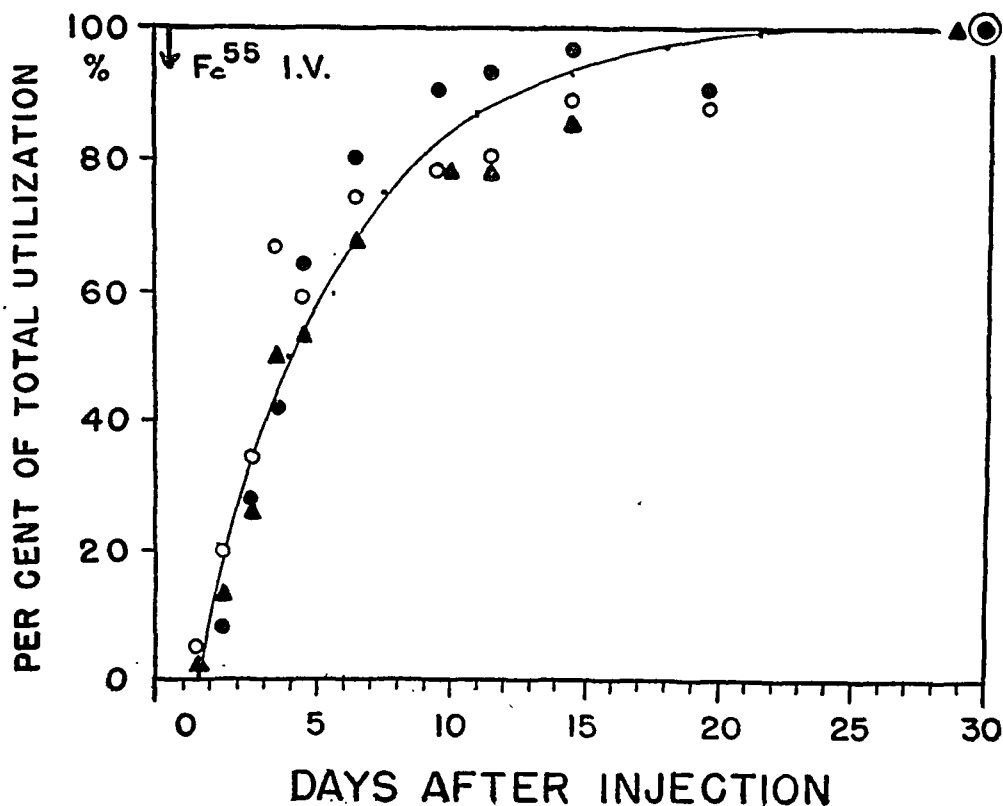

FIG. 1. UTILIZATION OF INTRAVENOUSLY INJECTED RADIOACTIVE IRON

The subjects were males in normal iron balance, and in all 3 approximately 80 per cent of the administered radio-iron was found in circulating red cells at the end of the observation period. The percentages of utilization plotted are normalized. The solid curve represents an exponential growth curve such that half of the radioactive iron available for red cell production is utilized every 3½ days.
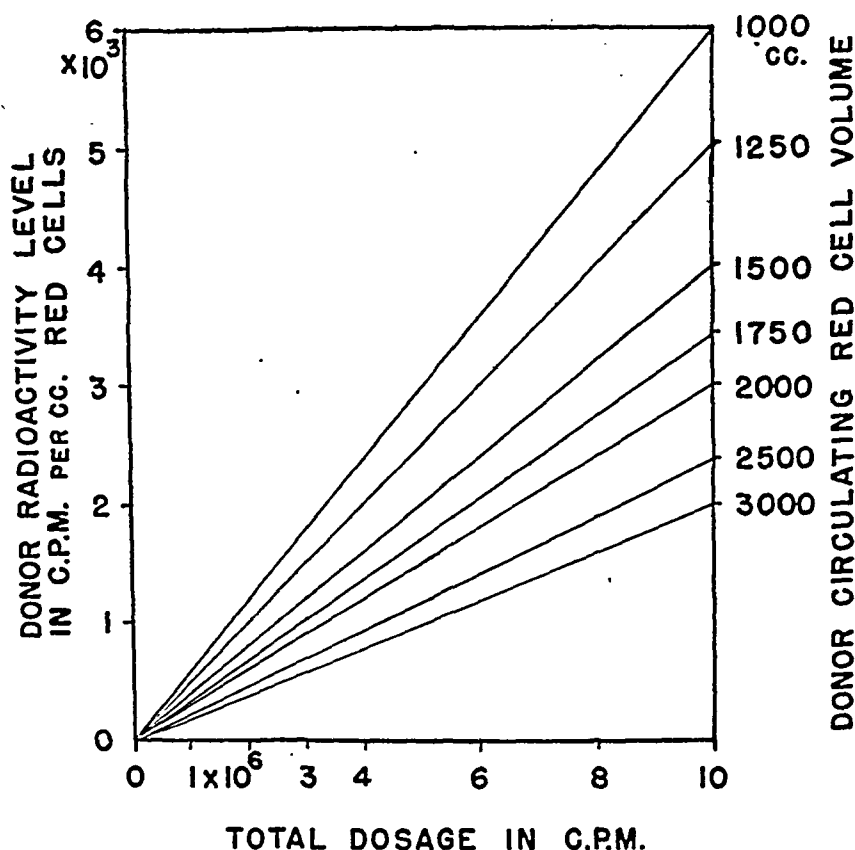
FIG. 2. DONOR RED CELL RADIOACTIVITY LEVELS

Nomogram for approximation of donor red cell radioactivity in c.p.m. per ml. of cells resulting from intravenous injection of radioactive ferric ammonium citrate in total dosage up to 1×10^7 c.p.m. (given either as a single or in multiple injections) in subjects whose total cell volume ranges from 1000 to 3000 ml.

Preparation of donor's red cells for infusion.

Red cells deteriorate rapidly at room temperature. It is essential that the donor blood be fresh if simple citrate solutions or heparin are used as anticoagulants. If the donor blood must be kept for more than 12 hours, it should be drawn into a preservative solution and kept at not over 10° C. until shortly before it is used. The best preservative so far encountered is an acid-citrate-dextrose solution having the formula:

	grams per 100 ml.
Tri-sodium citrate dihydride	1.33
Citric acid: H_2O	.47
Dextrose, anhydrous	3.00
pH	5.0

The components are dissolved together in pyrogen-free distilled H_2O and can be autoclaved without discoloration. Twenty-five ml. of this solution are used for taking 100 ml. of whole blood. It is advisable to chill the solution before taking blood into it. Small fibrin clots may form, requiring filtration. The pH of the solution is 5.0, and

though not isotonic, it does not hemolyze cells or caus appreciable changes in cell dimensions because of th rapid buffering action of plasma protein and hemoglobi Cells in this solution can be used for 5 days, since 90 t 95 per cent of them survive post-transfusion after 10 day of refrigerated storage. If fresh blood is used, filterin is unnecessary. The amount of blood given must be ac curately measured, and syringes or delivery burette should be calibrated to within 1 ml.

The sample of donor blood for radioactivity analysis taken at the time of infusion. If the blood is filtered, th sample is of the filtered blood. All donor blood is thor oughly mixed by gentle rotation just prior to administ tration.

The donor blood is injected into the blood stream vi vein, artery or auricle. After allowing 10 to 20 minute for complete mixing to take place, a blood sample is take usually followed by 2 others at 10 to 15 minute interval Samples are taken without stasis. We have routin taken 15 ml. of blood in round-bottomed graduated c trifuge tubes using heparin in the sampling syring an anticoagulant. If a dye-plasma volume is determ

simultaneously, the sampling time schedule can be so arranged that plasma from the radio-iron samples may be used for dye colorimetry.

If the subject has never received radio-iron, it is unnecessary to take a blood sample before infusing the donor's blood. Should a second injection of radioactive cells be given for the repeated determination of cell volume, it is necessary to take a blood sample just prior to the second infusion. This sample is essential if non-radioactive red cells are given between measurements.

Recipient blood samples should never be taken through needles or syringes, etc., with which donor's blood has come in contact since large and uncorrectable errors may result from radioactive contamination.

Successive cell volume determinations can be carried out. Each volume is calculated from the net increase in radioactivity level resulting from the last injection of cells.

Preparation of red cell samples for radioactivity measurement.

The electroplating of iron in preparation for counting has been described elsewhere (17). Only the procedures involved in wet-ashing the blood samples and precipitating the iron will be described here.

Blood donor samples.

In order that counting rates on both donor and recipient samples have approximately the same probable error, it is desirable that both donor and recipient sample be in the same range of counting rate. Donor cell levels are always higher than recipient levels, usually by a factor of from 40 to 50. The typical procedure is to dilute 2 ml. of whole donor's blood (using an Ostwald pipette) to 100 ml. in a volumetric flask and prepare 10 ml. aliquots of this for analysis in duplicate. Wintrobe hematocrits, preferably in duplicate, are determined for each donor whole blood sample, and the number of ml. of cells in the sample calculated from these values. Since recipient samples contain about 5 ml. of cells, donor and recipient radioactivity levels are approximately equal.

Blood recipient samples.

The 15 ml. graduated collecting tubes are centrifuged at 3000 r.p.m. for 30 minutes to obtain fairly complete packing. The number of ml. of cells is noted to the nearest 0.1 ml. The hematocrit of the blood sample may also be noted. The plasma is then removed.

Once the donor blood has been diluted, or the plasma removed from the recipient cells, the samples can be
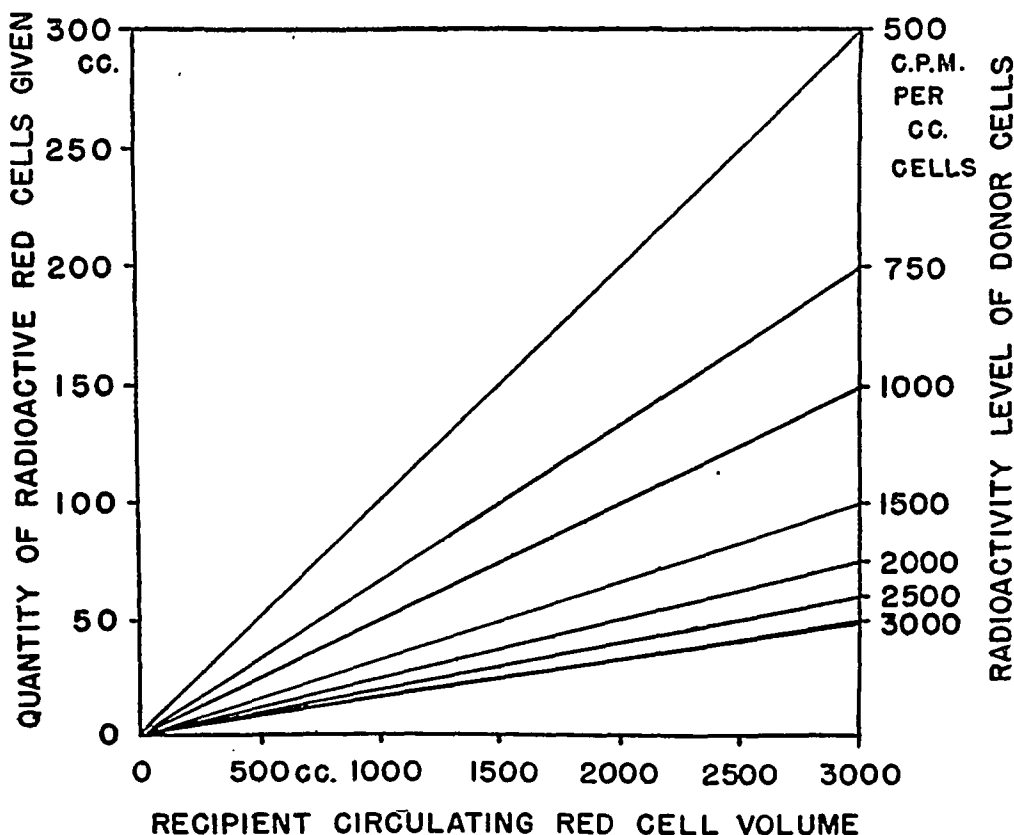

FIG. 3. RECIPIENT RADIOACTIVITY RED CELL LEVELS

Nomogram for computation of the minimum quantity of donor red cells, the radioactivity of which is from 500 to 5000 c.p.m. per ml. necessary to obtain a minimum recipient red cell activity of 50 c.p.m. per ml., in recipients whose total cell volume is between 500 and 3000 ml.

stored for considerable periods of time before wet-ashing. This permits of transporting or shipping samples either as laked or packed cells. Wet-ashing and precipitation of iron has been described in a previous communication (17).

The protocol of a typical human experiment,[3] in which

radio-iron red cell volume, dye plasma volume and total blood volume were measured before and after a large

New York City, in collaboration with Drs. Andre Cournand and Alice Lowell. The preparation for and radio-activity analyses of samples were carried out in our laboratories. We wish to express our gratitude for permission to use these data.

[3] This experiment was performed at Bellevue Hospital,

EXPERIMENT No. CR-1 1-22-45

$$V_{rr}{}^1 = \frac{50.0 \times 12.3}{0.208} = 2960 \, cc.$$

$$V_{rr}{}^2 = \frac{45.3 \times 13.7}{0.451 - 0.208} = 2560 \, cc.$$

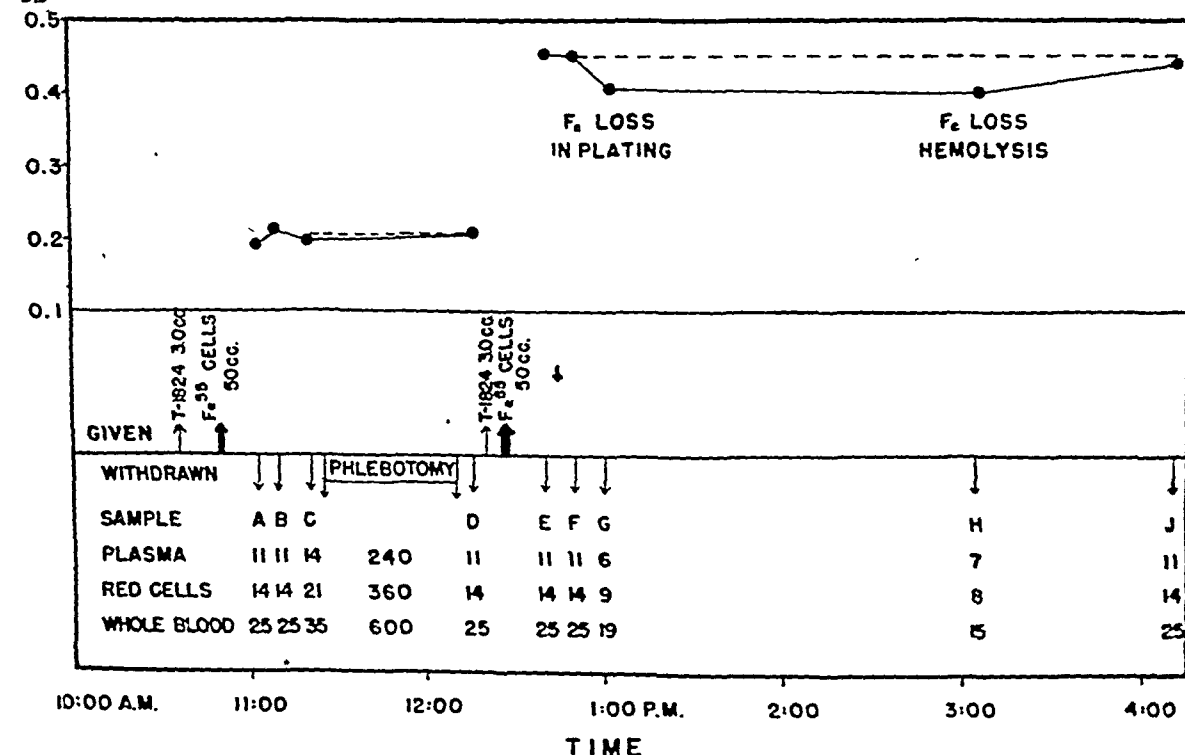|		INITIAL	NET LOSS	EXPECTED	FOUND
V_{rr}	cc.	2960	359	2601	2560
V_{pd}	cc.	2510	276	2234	2750
Fe^{55} U_a	TOTAL PROTEIN G.	168	18	150	180

FIG. 4. DETERMINATION OF CIRCULATING RED CELL VOLUME BEFORE AND AFTER PHLEBOTOMY BY MEANS OF RADIOACTIVE IRON

Fifty ml. of cells tagged with Fe[55] were injected for determination of the initial cell volume (V_{rr_1}). The U_a of these cells was 12.3, and the U_a of the recipient's cells after mixing averaged 0.208, making $V_{rr_1} = 2960$ ml. The net loss in cells from phlebotomy, and cells withdrawn in samples A–D inclusive was 359, so that the expected post-phlebotomy cell volume was 2601 ml. The recipient's cell U_a was not altered by the phlebotomy, indicating *complete mixing* of the tagged with the recipient's cells. 45.3 ml. of cells tagged with Fe[55] were injected for the post-phlebotomy cell volume (V_{rr_2}) determination. As a result, the recipient's cell U_a rose to an average value of 0.451 (values of samples G and H in which loss of iron in processing was known to have occurred were discarded), or $V_{rr_2} = 2560$ ml., i.e., 41 ml. of cells less than the expected cell volume, an error of + 1.6 per cent.

phlebotomy, follows. The significant data are shown in Figure 4.

Experiment No. CR-1. January 20, 1945.

Subject: F. D. Age 62. Height, 63.5 cm. Weight, 70.5 kgm.

Diagnosis: Emphysema with secondary polycythemia.

The patient lay in bed in a semi-reclining position throughout the experiment. All blood samples were taken through an indwelling cannula in the femoral artery. Dye and radioactive cells were given into a median cephalic vein. Patient was bled from the femoral artery.

Radioactive cells were obtained from a previously prepared patient with polycythemia and were group-O. Since the donor's serum anti-A titre was not known, his blood was taken in 4 per cent sodium citrate, centrifuged, the plasma removed and the cells resuspended in a volume of isotonic citrate-saline solution equal to the removed plasma. Patient experienced no untoward subjective symptoms.

Red cell volumes were calculated from the formula

$$Vrr = \frac{CD \times UaD}{UaR},$$

where Vrr is the red cell volume by radio-iron, CD is the number of ml. of donor cells given, UaD the radioactivity of the donor's cells, and UaR the radioactivity of the recipient's cells. Then

$$Vrr^1 = \frac{50.2 \times 12.3}{.208} = 2960 \text{ ml.}$$

and

$$Vrr^2 = \frac{45.3 \times 13.7}{.451 - .208} = 2560 \text{ ml.}$$

The average Ua of the recipient's samples taken after each infusion of cells has been used. Values known to be low due to observed loss of iron in plating and to hemolysis were discarded, as was the venous sample CR 1j. Vrr^2 was calculated from the *net* Ua level due to the second infusion of cells.

The dye plasma and radio-iron cell volume, measured before and after phlebotomy, are given in Table IV. For purposes of comparison, the red cell volume calculated from the dye-plasma-hematocrit is also given.

This protocol illustrates several points. The value for Vrr^1 includes the patient's total red cell volume, and also the radioactive cells given for determination of that volume. Vrr^2 includes the patient's residual cells after bleeding, plus the second infusion of radioactive cells minus cells removed in sampling after the first infusion.

The quantity of cells withdrawn by phlebotomy was measured by the radio-iron technique with an error of − 2.2 per cent. At both volume measurements Vrr was less than Vrpd, by 22 and 26 per cent respectively. The calculated dye-plasma-hematocrit red cell volumes did however measure known red cell loss with considerable accuracy. The patient added 12 grams of protein to his circulation after hemorrhage and there was an increase of 510 ml. of plasma over the expected values. The arterial hematocrit was lowered by this hemodilution, but not to as great an extent as was the average body hematocrit.

Experiment No. CR-1
January 20, 1945

| Time | Procedure | Sample no. | Blood withdrawn | | | | Radioactivity |
			Whole blood	Hct.	Plasma	Cells	
			ml.	*per cent*	*ml.*	*ml.*	*Ua*
10:36 A.M.	3.0 ml. of 0.5 per cent T.1824 i.v. Syringe 4695-Y						
10:48	100 ml. of suspended	CD 1a		50.2			12.5
10:51	radioactive cells i.v.	CD 1b		49.7		50	12.1
11:02	Sample ⎫ for dye plasma	CR 1a	25	60.3	11	14	0.191
11:09	Sample ⎬ volume and	CR 1b	25	59.3	11	14	0.212
11:21	Sample ⎭ radioactivity	CR 1c	35	59.8	14	21	0.197
11:25	600 ml. of whole blood						
12:05 P.M.	withdrawn from femoral artery at av. Hct. of 60 per cent			60	240	360	
12:16	Samples	CR 1d	25	57.7	11	14	0.206
12:19	3.0 ml. of 0.5 per cent T.1824 i.v.						
12:25	100 ml. of suspended						
12:28	radioactive cells i.v.	CD 1b		45.3			13.5
	Syringe 4891-Y	CD 1c		45.2		45	13.9
12:40	Sample ⎫	CR 1e	25	56.6	11	14	0.452
12:50	Sample ⎬ for dye plasma	CR 1f	25	55.7	11	14	0.450
1:00	Sample ⎬ volume and	CR 1g	15	57.7	6	9	0.405*
3:03	Sample ⎬ radioactivity	CR 1h	15	54.3	7	8	0.400**
4:12	Sample† ⎭	CR 1j	25	57.2	11	14	0.438

* Sample hemolyzed.   ** Some iron loss in plating.   † Venous blood.

TABLE IV

Measurement of plasma (dye) and red cell volume (radio-iron) in a patient with secondary polycythemia before and after a large phlebotomy*

	Plasma volume	Directly measured by radio-iron		Calculated from dye-plasma-hematocrit		Arterial hematocrit	Average body hematocrit	$R = \dfrac{Vrr}{Vrpd}$
	Vpd	Vrr	Vwr	Vrpd	Vwpd	Hav	Hb	
	ml.	*ml.*	*ml.*	*ml.*	*ml.*	*per cent*	*per cent*	
Before phlebotomy	2510	2960	5470	3740	6250	59.8	53.8	0.79
**Plasma and red cell loss (direct measurement)	286	359	645	313	599			
†Calculated volumes after bleeding	2224	2601	4825	3427	5651			
Measured volume after bleeding	2750	2560	5310	3510	6260	56.1	48.3	0.73

Symbols: Vpd = Dye plasma volume; Vrpd and Vwpd = red cell and total blood volume calculated from plasma volume and hematocrit.

Vrr = Red cell volume measured by radio-iron.

Vwr = Total blood volume = Vpd + Vrr.

Hav = Large vessel hematocrit (in this case arterial).

Hb = $\dfrac{Vrr}{Vwr}$ or average body hematocrit.

* Experiment No. CR-1, Bellevue Hospital, January 23, 1945.

** Includes blood taken from samples.

† Corrected for second transfusion of radioactive cells.

COMMENT

The specificity and objectivity of the method described make it a desirable research tool in clinical investigation.

We have applied the method, with suitable modifications, to the study of a wide variety of experimental conditions. The normal red cell volume in humans and dogs has been studied. We have investigated shock experimentally produced · by hemorrhage, muscle crushing, arterial occlusion, burns and bacillary toxins. The results have thrown light on many aspects of circulatory collapse. In the course of following changes in total cell volume in shock, it became evident that the withdrawal of red cells from active circulation was characteristic of severe collapse. The technique has been applied in a manner designed to directly measure the quantities of both plasma and cells normally coursing through the minute vessels, and the extent to which cells are trapped in the capillaries of the several vital organs in deep shock.

The method has also been adapted to the measurement of post-transfusion survival of human erythrocytes stored, both as whole blood and packed cells, in a number of preservative solutions. Findings are now in preparation for reporting.

These studies have been conducted in collaboration with many investigators, and will be reported in later communications.

SUMMARY

1. A method of determining circulating red cell volume by means of either of two radioactive isotopes of iron, based on the original technique of Hahn and associates, is described.

2. The method gives an absolute measure of the *circulating* red cells, which is independent of the venous, arterial or auricular hematocrit.

3. Repeat red cell volume measurements can be determined without errors arising from residual red cell radioactivity levels.

4. When used in conjunction with the dye plasma volume methods, the method provides an accurate measurement of circulating total blood volume.

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A CLINICAL STUDY OF TRANSFUSION REACTIONS: THE HEMO-LYTIC EFFECT OF GROUP-O BLOOD AND POOLED PLASMA CONTAINING INCOMPATIBLE ISOAGGLUTININS

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The stored blood employed during World War II for the treatment of battle casualties was almost exclusively group-O blood, in consequence of which patients of blood groups A, B and AB were obliged to receive incompatible isoagglutinins in the course of transfusion. Anti-A and anti-B isoagglutinins, incompletely absorbed in the process of pooling, were likewise contained in the dried plasma supplied by the Army. Thus, all patients of blood groups other than group O receiving pooled plasma, and over 50 per cent of those who were transfused with whole blood were subjected to the injection of incompatible iso-agglutinins.

The preponderance of evidence in the literature indicates that the transfusion of group-O blood into recipients of other blood groups rarely produces serious reactions (1). The most extensive observations in this connection were reported by Aubert *et al.* (2), who described instances of chills, fever and evidences of blood destruction, but no serious complications following such transfusions. Reports of severe reactions in response to the injection of incompatible group-O blood have, however, appeared from time to time. A proper evaluation of such records in the earlier literature is difficult, because of the lack of data pertaining to Rh compatibility, but isolated cases have more recently been described (3 to 5) in which the Rh factor clearly could not be implicated. The present report is based on a detailed study of transfusion recipients, including 85 soldiers who received incompatible isoagglutinins in varying amounts. This investigation has been conducted for the purpose of determining in what manner and to what extent humans may react to the administration of whole blood and plasma containing these isoagglutinins, with the hope that such observations might yield data of practical

significance pertaining to the practice of transfusion therapy, or, possibly, of theoretical interest in relation to hemolytic processes in general.

MATERIALS AND METHODS

The sera of 184 blood donors were examined in order to estimate the relative frequency with which individuals of group-O blood are encountered who exhibit a high titer of isoagglutinins. These donors, selected at random, were all healthy adult males and females. Pooled washed erythrocytes of groups A and B were employed as the test cells, prepared in the form of a 2 per cent suspension in normal saline solution. Agglutination was read grossly and microscopically after centrifuging the test preparations. The titer values are expressed in terms of the final dilution following addition of the cell suspension, and the incidence of occurrence is recorded in Table I. Isoagglu-

TABLE I

Incidence of isoagglutinin titers determined in 184 group-O blood donors

Titer	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
	Per cent of donors							
Anti-A	0	5	10	30	25	17	11	2
Anti-B	0.5	9	16	29	25	17	3	0.5

tinin titers, determined in 5 lots of reconstituted pooled dried human plasma, selected at random, ranged from 1:32 to 1:128.

Differential counts of agglutinable and non-agglutinable erythrocytes in the blood were performed by the method of Ashby (6), modified to permit the use of dried anti-A and anti-B grouping sera (Lederle), a step which eliminated the factor of dilution by serum. The mixture of dried serum and cell suspension was centrifuged for 1 minute and subsequently allowed to stand for 5 minutes, following which the cells were resuspended. The non-agglutinated cells were counted in a standard hemocy-tometer, and this count compared with a similar count done on the cell suspension to which no grouping serum had been added. The reliability of this method was established through control counts performed on preparations containing O and A cells in known proportions.

Measurements of the plasma hemoglobin concentration were performed by the method of Ring and Baker (7).

modified for the photoelectric cell colorimeter. Careful precautions were observed in obtaining plasma specimens for this determination. Three ml. of blood were removed from a vein, using a 10 ml. syringe moistened with 2½ per cent sodium citrate solution, and introduced into a small test tube containing 0.4 ml. of 2½ per cent sodium citrate solution. The tube was then stoppered with a waxed cork and inverted once. Immediately thereafter it was centrifuged slowly for 5 minutes; the supernatant fluid was again centrifuged rapidly for 5 minutes, and this supernatant material employed for the determination. With this technique it was possible consistently to obtain values for plasma hemoglobin less than 2 mgm. per cent in normal subjects. In determining the concentration of serum or plasma bilirubin in specimens containing free hemoglobin, a correction factor was employed to eliminate an additive error due to the presence of hemoglobin.

Serum bilirubin concentrations were determined by the Van den Bergh method (8), modified for the Klett photo-electric cell colorimeter and standardized with purified bilirubin. The total hemoglobin concentration was measured colorimetrically with the Klett colorimeter. Hematocrits were read in 4 ml. tubes centrifuged at 2000 r.p m. for 30 minutes, mixed ammonium and potassium oxalate being used as the anticoagulant. The method for estimating the osmotic fragility of erythrocytes depended upon the measurement of the percentage of total hemoglobin liberated by hemolysis in a mixture of 1 part whole blood with mixed oxalate, and 50 parts of the sodium chloride test solution, the hemoglobin concentration in the supernatant solution being determined with the Klett colorimeter, and the percentage hemolysis, calculated therefrom, being charted with relation to the concentration of sodium chloride.

The plasma volume measurements were performed by the method of Gibson and Evelyn (9), modified to permit the determination of the dye concentrations in oxalated plasma (10), using the Klett colorimeter. In computing the red cell volume from the figures obtained for the plasma volume and hematocrit reading, a standard correction has routinely been applied, based on evidence (11, 12) that a systematic additive error of 15 per cent is inherent in this calculation.

The anticoagulant employed for all transfusions was sodium citrate.

Rh grouping tests reported in this communication were performed with potent human anti-Rh serum supplied through the kindness of Dr. Louis K. Diamond, Blood Grouping Laboratory, Boston, Mass.

RESULTS

Transfusion reactions occurring after the administration of blood containing incompatible isoagglutinins in high titer.

A few reactions were observed in patients receiving therapeutic blood transfusions, the explanation for which appeared to be related to the presence of a high concentration of incompatible isoagglutinins in the transfused blood. An instance of this type of reaction is given:

The patient, whose blood was group-A Rh + had received a gunshot wound of the right leg, following which he had been given 3 transfusions of whole blood. Two weeks later he received a transfusion of fresh group-O blood. Twenty minutes after the transfusion was started, 350 ml. of blood having been injected, he began to complain of back pain, nausea, vomiting and chilliness. The transfusion was promptly halted. A specimen of urine obtained immediately after the reaction contained a moderate amount of hemoglobin, but subsequent samples were hemoglobin-free, and urine secretion remained undiminished. The plasma hemoglobin concentration immediately after the transfusion was 86 mgm. per 100 ml.; two hours later it was 32 mgm. per 100 ml. The serum bilirubin concentration at the conclusion of the transfusion was 1.6 mgm. per 100 ml., at the end of two hours it was 3.7 mgm. per 100 ml., and after twelve hours it was 1.3 mgm. per 100 ml.

The patient recovered uneventfully, and subsequently received several transfusions of both group-O and group-A blood without further incident. Studies confirmed that the blood of the donor was group-O, and that of the recipient was group-A. The donor cells were not agglutinated or hemolyzed *in vitro* by the serum of the recipient, either at room temperature or after incubation at 37° C. The titer of anti-A isoagglutinins in the plasma of the transfused blood was 1:2048. Ashby counts indicated that, prior to transfusion, 58 per cent of the patient's red cells were non-agglutinable, evidence that the previous transfusions had been of group-O blood. Twelve hours after the transfusion reaction the proportion of non-agglutinable cells was 75 per cent, indicating that the transfused cells almost certainly were not involved in the hemolytic process.

Interest in the features presented by this case prompted a subsequent investigation, conducted in an Evacuation Hospital, designed to study the incidence and clinical manifestations of this type of reaction. During the period of study, 265 transfusions of group-O blood were administered to 61 patients of groups A, B, or AB. There occurred 3 reactions which could be related to a high titer of incompatible isoagglutinins in the transfused material, representing an incidence of 1.1 per cent. The titer of isoagglutinins in the blood implicated in these reactions ranged from 1:500 to 1:1500, when the donor plasma was tested against the recipient cells. No similar reactions were observed with titers of less than 1:500.

The overt clinical manifestations of these reactions consisted of a brief chill and a moderate

febrile response. Hemoglobinuria and marked hemoglobinemia were observed in one case. A transient slight elevation of plasma hemoglobin concentration was present in the other cases, but no hemoglobinuria developed. Hyperbilirubinemia, lasting from 12 to 24 hours, was consistently present, with icterus index elevations in no case exceeding 3 or 4 times the normal value. Serial Ashby counts indicated that the transfused cells were not destroyed with unusual rapidity; on the contrary, they could be demonstrated in unexpectedly high proportions in the blood of these patients following transfusion, suggesting that recipient red cells rather than donor cells had been destroyed.

Control studies were carried out in order to determine the effect of injecting incompatible plasma, in the absence of donor cells. Four healthy adult male volunteers, previously untransfused, whose blood groups were A or B, received single rapid intravenous injections of plasma derived from group-O blood, the isoagglutinin titers

of which had been demonstrated to be unusually high. The titers determined in these plasma preparations, tested against the recipient red cells, in 1 case was between 1:500 and 1:1000, and, in the remaining instances, between 1:2000 and 1:4000. The volume of plasma injected ranged from 250 ml. to 500 ml.

No clinical symptoms were produced as a result of these transfusions, and only 1 subject exhibited a slight transient febrile response, the oral temperature rising to 99.3° F. The effects of the injections on the levels of serum bilirubin, plasma hemoglobin and total hemoglobin concentrations are illustrated in Figure 1. In each case a slight rise in serum bilirubin concentration occurred, attaining a maximum in from 2 to 6 hours, and gradually subsiding to normal over a period of 12 to 24 hours. In 2 cases the plasma hemoglobin concentration rose to 10 mgm. per 100 ml., the peak concentrations developing within 30 minutes following the plasma injection, and the hemoglobinemia clearing completely in from 6 to 12
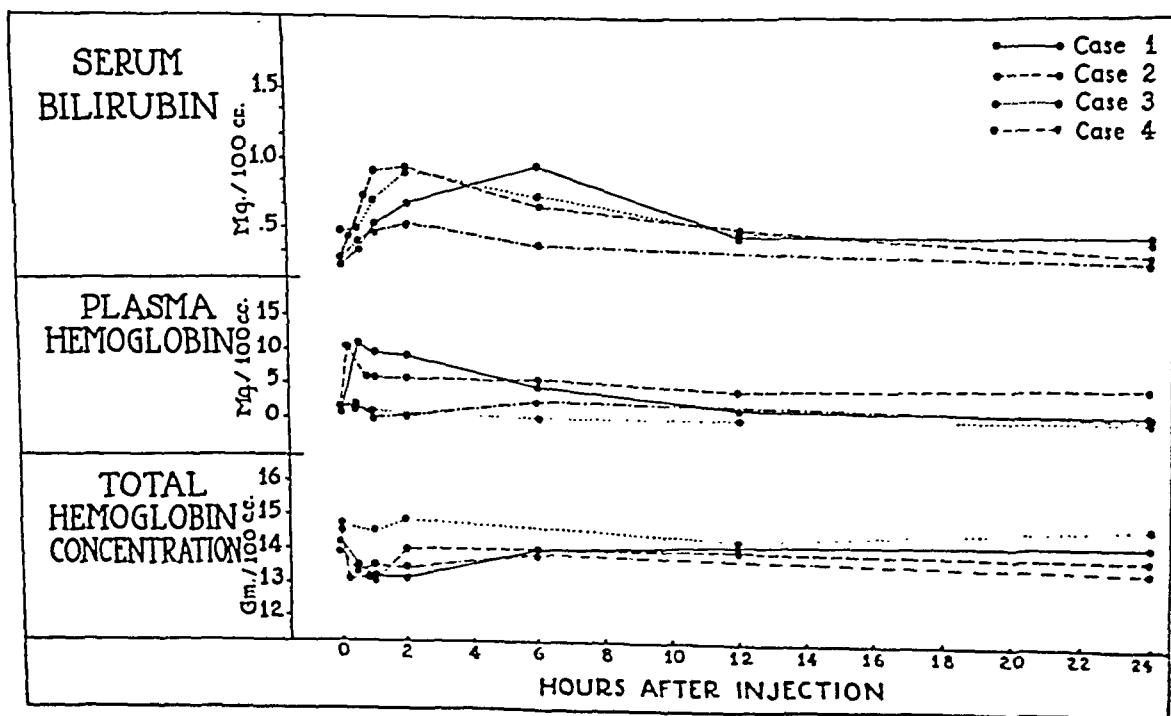

FIG. 1. THE EFFECT OF PLASMA CONTAINING A HIGH CONCENTRATION OF INCOMPATIBLE ISOAGGLUTININS, WHEN INJECTED INTO NORMAL HUMAN SUBJECTS

Two hundred and fifty to 500 ml. of plasma containing isoagglutinins in titers ranging from 1:500 to 1:4000 against the recipient cells were injected into four subjects whose blood groups were A or B. Note the definite increase in serum bilirubin and slight or absent elevation of plasma hemoglobin concentration

hours. No significant elevation of plasma hemoglobin concentration was observed in the remaining cases, and in no instance was hemoglobinuria encountered. The total hemoglobin concentration showed only a slight initial decline, consistent with the anticipated effect of erythro-dilution due to the injected plasma.

The results of these experimental plasma transfusions appeared to correspond qualitatively with the observations made in cases receiving therapeutic transfusions of whole blood containing a high concentration of incompatible isoagglutinins. To be sure, none of the 4 individuals receiving cell-free plasma exhibited a chill or other significant clinical symptomatology; nor was the apparent degree of blood destruction, judged on the basis of hemoglobinemia and bilirubinemia, equivalent to that occurring in some of the clinical cases

in which there was a febrile response to group-O whole blood, despite the fact that the plasma recipients were presumed to have received as high a dosage of incompatible isoagglutinins as did those who reacted to the injection of whole blood. Whether this apparent disparity in quantitative effect may be attributable to the presence of red cells in the donor blood, or to an enhanced susceptibility on the part of those patients, was not determined. It is possible that the *in vitro* estimation of relative isoagglutinin potency is an inadequate measure of the hemolytic property of the material when introduced into the recipient.

The effects of single injections of cell-free hemoglobin solution.

In contrast to the findings in certain other hemolytic syndromes, the degree and duration of
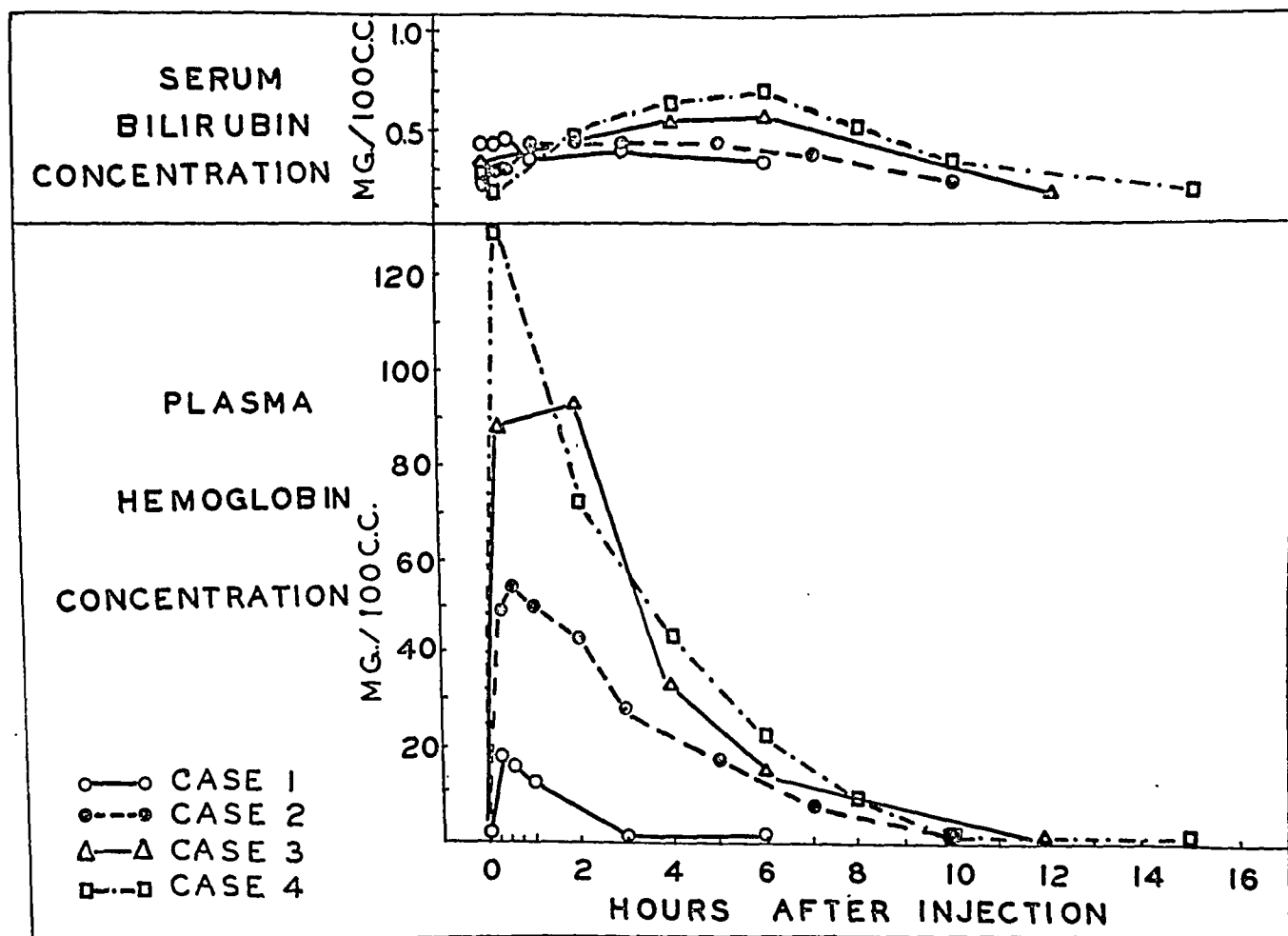

FIG. 2. THE EFFECT OF INJECTING HEMOGLOBIN SOLUTION INTO NORMAL HUMAN SUBJECTS

Case 1 received 1.2 grams of hemoglobin intravenously; Case 2, 2.4 grams; Case 3, 4.0 grams; and Case 4, 6.2 grams. Note the relatively minor effect of these injections on the serum bilirubin concentration, in proportion to the marked hemoglobinemia produced.

hyperbilirubinemia following the transfusion of incompatible isoagglutinins appeared to be disproportionately great compared to the degree of hemoglobinemia. A soldier with march hemoglobinuria, for example, was observed to have a plasma hemoglobin concentration of 40 mgm. per 100 ml. following a march which had induced hemoglobinuria; at the end of a 3-hour period of rest, at which time the urine was hemoglobin free, the plasma hemoglobin concentration was still approximately 10 mgm. per 100 ml. However, the maximum concentration of serum bilirubin, attained one hour following the march, was only 0.85 mgm. per 100 ml. A more striking clinical example illustrating the occurrence of marked hemoglobinemia, not followed by significant hyperbilirubinemia, was afforded by the case of a transfusion accident with recovery. Through error, a patient of blood group-O received 120 ml. of group-A blood. One hour following transfusion the plasma hemoglobin concentration was 240 mgm. per 100 ml.; 8 hours later it was still 30 mgm. per 100 ml.; nevertheless, the peak level

of bilirubinemia was less than 1.0 mgm. per 100 ml. These inconsistencies of relationship between the concentrations of plasma hemoglobin and bilirubin prompted a series of experiments in which normal human subjects received varying amounts of cell-free hemoglobin intravenously, serial measurements of the plasma hemoglobin and serum bilirubin concentrations being performed following injection.

The recipients were normal young adult males who had volunteered to serve as subjects for these experiments. Hemoglobin solutions were prepared by lysing, in distilled water, a citrated specimen of whole blood obtained from the prospective recipient. The isotonicity of the hemolysate having been restored and the red-cell stroma precipitated by the addition of crystalline sodium chloride, the material was prepared for injection by passage through a Seitz filter. Injection of these solutions in no instance induced a febrile response or other unfavorable sequelae. Transient hemoglobinuria occurred in 1 subject (Case 4) who received 6.2 grams of hemoglobin, the lar-

TABLE II

The effect of group-O donor blood on the total volume of recipient red cells, in patients of blood groups A and B

Case (blood group)	Volume of group-O blood transfused	Time	Hematocrit	Plasma volume	Non-agglutinable red cells	Volume of group-O cells transfused	Change in volume* of	
							Group-O cells	Group-A or B cells
	ml.			*ml.*	*per cent*	*ml.*	*ml.*	*ml.*
1 (ARh+)	1120	before transfusion	30.8	2880	1	470	450	−430
		48 hr. after transfusion	28.5	3270	41			
2 (ARh+)	1560	before transfusion	31.2	2700	19	640	700	−560
		48 hr. after transfusion	34.8	2600	76			
3 (ARh+)	1000	before transfusion	34.3	2670	0	490	390	+130
		20 hr. after transfusion	38.3	3230	23			
4 (ARh+)	1460	before transfusion	35.4	3090	8	640	620	−170
		72 hr. after transfusion	41.4	3140	39			
5 (BRh+)	1100	before transfusion	32.4	3060	12	470	460	+80
		24 hr. after transfusion	40.1	3160	34			
6 (A)	1000	before transfusion	36.3	2760	1	390	340	−70
		24 hr. after transfusion	40.5	2780	22			
7 (ARh+)	1020	before transfusion	40.1	2940	47	420	350	−40
		48 hr. after transfusion	44.8	2880	57			

* Calculated from plasma volume, hematocrit and percentage of non-agglutinable R.B.C. before and after transfusion.

gest dose administered. The effects of these injections on the concentrations of plasma hemoglobin and serum bilirubin are illustrated in Figure 2.

It will be noted that, in common with the situation following the transfusion of incompatible red cells, and in contrast to the findings determined in cases receiving incompatible plasma, only minimal elevations of bilirubin concentration occurred in conjunction with relatively marked and sustained hemoglobinemia. The inference may be drawn, that the hemolytic process induced by the injection of incompatible plasma does not involve a rapid intravascular destruction of the recipient erythrocytes with the liberation of corresponding amounts of free hemoglobin into the free circulation, for the amount of bilirubin produced under these conditions is greater than could be accounted for, assuming its sole source to be the hemoglobin circulating in the plasma.

The hemolytic properties of incompatible group-O blood and pooled plasma.

Despite the infrequent occurrence of febrile reactions following the injection of incompatible isoagglutinins, it was suspected that asymptomatic hemolysis of recipient cells may be a common complication of this procedure. In order to evaluate this possibility and to determine quantitatively the degree of hemolysis produced, measurements of the blood volume, together with Ashby counts, were performed before and after a single transfusion of group-O whole blood in 7 recipients whose blood groups were A or B. None of these patients had experienced recent hemorrhage. All were battle casualties convalescing from chest wounds, with the exception of Case 1, who was a patient with subacute glomerulonephritis.

The results of these studies are charted in Table II. It is evident that a significant degree
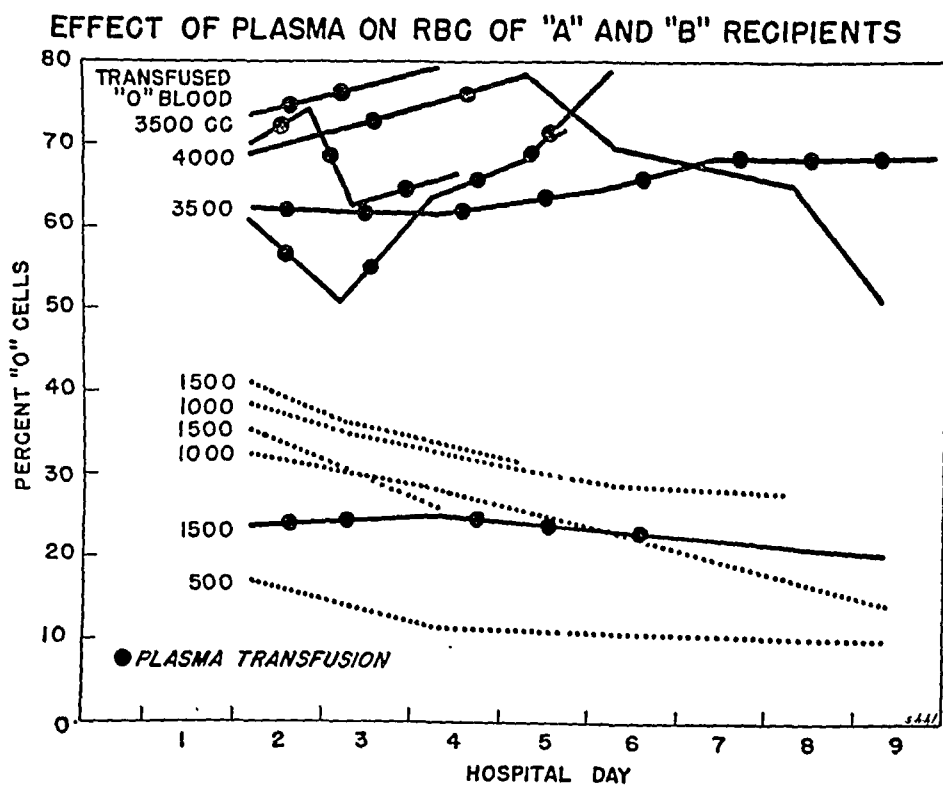

FIG. 3. THE DESTRUCTIVE EFFECT OF REPEATED PLASMA TRANSFUSIONS ON THE ERYTHROCYTES OF GROUP-A AND GROUP-B RECIPIENTS

All patients received an initial transfusion of stored group-O blood, the amount of which is indicated. The broken lines represent patients who received no further transfusions; the solid lines represent patients who subsequently received transfusions of pooled plasma, each transfusion being indicated by a dot. A progressive decline in the percentage of group-O cells is observed in the former group. An increase in this percentage indicates destruction of the recipient red cells, which is more rapid than the disappearance of the transfused cells in patients receiving pooled plasma.

of blood destruction involving the recipient cells followed transfusion in Cases 1, 2 and possibly 4. Such variations as are noted in the other cases, with respect to the changes in red cell volumes, are within the limits of experimental error. It is noteworthy that in no case, including those in whom a considerable degree of blood destruction occurred, was transfusion followed by chills or fever.

Blood or plasma containing incompatible isoagglutinins appeared to have a more destructive effect when administered in multiple repeated transfusions, as evidenced by the results of a study in which serial Ashby counts were performed over a period of several days in patients of blood groups A and B. The therapy in each case included an initial large transfusion of group-O blood, which had previously been stored for approximately two weeks. Whole blood transfusion was not repeated during the period of these observations. One group of patients received repeated plasma transfusions on successive days, while the other group,

which served as the control series, received no plasma during this period. The results are illustrated in Figure 3.

It was observed that, in cases receiving no plasma transfusions, the percentage of group-O cells progressively declined from day to day, the rate of their disappearance being consistent with the expected rate of destruction of injected, 2-weeks-old red cells. In contrast to the findings in these cases, however, those patients receiving repeated plasma transfusions failed to demonstrate the expected decline in percentage of group-O cells and, indeed, usually exhibited a persistent increase as long as this therapy was continued. It appeared, therefore, that the plasma recipients incurred a destruction of their own erythrocytes, which, in rapidity, equalled or exceeded that involving the transfused stored cells. More extended laboratory study of these patients, including serial measurements of the icterus index and hematocrit readings, demonstrated the development of progressive anemia and hyperbilirubine-
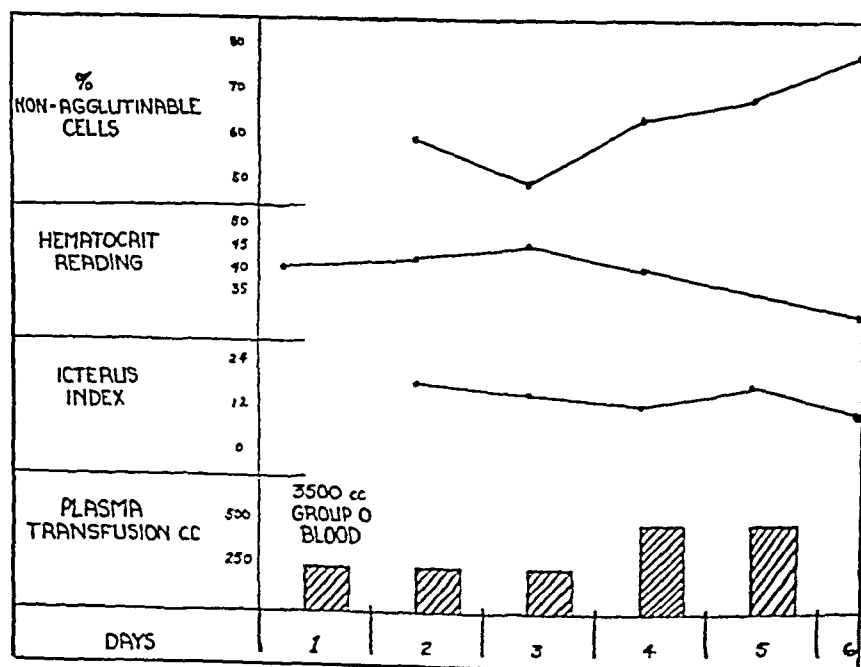

FIG. 4. THE HEMOLYTIC EFFECT OF POOLED PLASMA, OBSERVED IN ONE PATIENT OF BLOOD GROUP A

This figure illustrates the course of the Ashby count, hematocrit reading and icterus index in a patient of blood group A who received repeated transfusions of pooled plasma. Note the increasing percentage of group-O cells, indicating that rapid destruction of group-A cells has contributed to the development of anemia and persistence of icterus.

mia corresponding in degree with the severity of the hemolytic process as indicated by the Ashby counts (Figure 4).

Changes in the osmotic fragility of recipient erythrocytes following transfusions of plasma and whole blood containing incompatible isoagglutinins.

An unusual opportunity to study the effects of massive doses of pooled plasma on recipients of different blood groups was afforded in the course of observing 7 patients, who were treated for extensive gasoline burns. Three of these patients were of blood group-O. · The burns were of comparable degree and extent in all cases, and all received large amounts of pooled dried human plasma, supplemented, in some instances, by group-O whole blood. Each patient received a local application of sulfanilamide powder as part of the initial treatment of the burn, but in no case were sulfonamides administered by mouth or parenterally, by injection.

One feature of interest was the finding of a definite increase in the osmotic erythrocyte fragility in all cases, with the exception of those patients of blood group-O. The fragility curves, determined 48 to 72 hours after injury, are charted in Figure 5, with a notation of the patients' blood groups (O), (A) and (AB), as well as the amount of plasma (P) and whole blood (WB) received prior to the test. Ashby counts, carried out in saline solutions of various concentrations, indicated that the increase in osmotic fragility involved only the recipient cells. The patient showing the most striking change in osmotic fragility (Figure 5) died on the 4th hospital day with an acute hemolytic anemia, progressively marked hemoglobinemia and hemoglobinuria having been present for 24 hours. In addition to 7000 ml. of pooled plasma, he had received 2500 ml. of group-O whole blood prior to death; at that time his hematocrit reading was 24, and 90 per cent of his circulating red cells were group-O cells, indicating almost complete destruction of his own erythro-
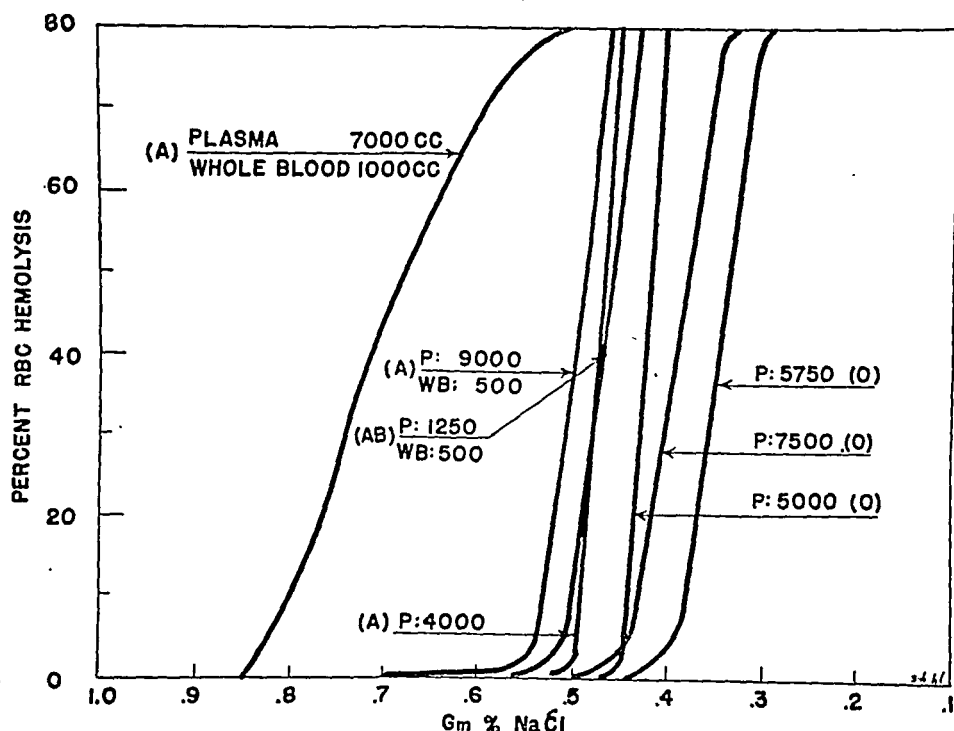

FIG. 5. OSMOTIC ERYTHROCYTE FRAGILITY AFTER TRANSFUSION THERAPY IN SEVEN PATIENTS WITH SEVERE BURNS

The blood group of each patient, together with the amount of plasma (P) and whole blood (WB) received, are indicated with each fragility curve. The whole blood used was exclusively group-O. Note the increased osmotic erythrocyte fragility in patients whose blood groups were other than group-O.
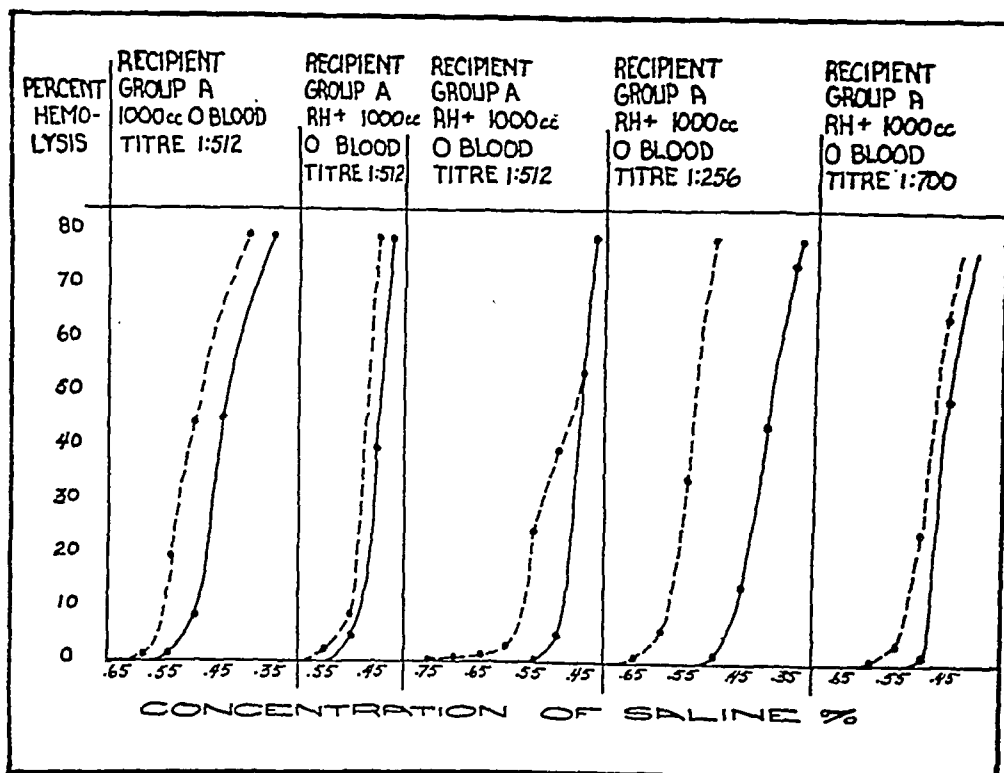
FIG. 6. CHANGES IN OSMOTIC ERYTHROCYTE FRAGILITY IN PATIENTS OF BLOOD GROUP A FOLLOWING TRANSFUSION OF GROUP-O BLOOD CONTAINING ANTI-A ISOAGGLUTININS IN HIGH TITER

The solid lines represent the osmotic fragility curves determined before the transfusion; the dotted lines, the fragility immediately following this procedure. The volume and isoagglutinin titer of the transfused blood are indicated in each case.

cytes. Combined Ashby counts and osmotic fragility tests further showed that all of his remaining group-A cells hemolyzed in concentrations of sodium chloride greater than 0.6 gram per cent, whereas the group-O cells, derived from transfusions, only began to hemolyze in solutions of less than 0.5 gram per cent sodium chloride. Another of the group-A patients demonstrated almost complete replacement of his own red cells by transfused cells, having received 10,000 ml. of pooled plasma and 6000 ml. of group-O whole blood in the course of 12 days. As in the case of the first patient described, his blood also exhibited a rapidly progressive increase in osmotic fragility affecting only his own red cells, as well as hemoglobinemia and hemoglobinuria for 2 days prior to death, which occurred on the 12th day following injury.

In order to determine whether these changes in osmotic fragility were, in fact, attributable to transfusion therapy rather than to the effects of the burn itself, or to other therapy accorded these patients, serial observations were made of the red cell osmotic fragility of 12 patients of blood group-A Rh + before and after transfusion with group-O blood containing anti-A agglutinins in various concentrations. Seven patients who received blood, the anti-A agglutinin titer of which exceeded 1 : 200, showed a definite increase in osmotic fragility which was demonstrable promptly after transfusion. Five patients receiving agglutinins in lower titer showed no fragility changes; one of these, however, following a subsequent transfusion of low-titer blood, exhibited a markedly increased osmotic fragility of the red cells. Similar studies carried out on group-O recipients indicated that no change in osmotic fragility occurred in these individuals following transfusion.

The effect of transfusion on the erythrocyte osmotic fragility in 5 group-A recipients of group-O blood is illustrated graphically in Figure 6. The dotted line represents in each instance the fragility

before transfusion, and the solid line the fragility determined immediately following its conclusion. All of the patients whose fragility curves are charted in this illustration had received previous transfusions of group-O blood and pooled plasma, which probably accounts for the deviations from the normal apparent in the pre-transfusion samples, inasmuch as similar abnormalities were never observed in individuals who had received no transfusions, or in patients of blood group-O. The irregular configuration of certain of these fragility curves is due to the fact that the blood examined contained a mixture of recipient cells and transfused group-O cells. This phenomenon of increased osmotic fragility of the red cells was demonstrable immediately following transfusion of blood containing a high concentration of incompatible agglutinins; the change was progressively more marked with repeated transfusions of this type; and, finally, the results suggested that red cells so affected remained abnormally susceptible to hemolysis in hypotonic solutions of sodium chloride until they eventually disappeared from the circulation.

DISCUSSION

It is concluded from these studies that the transfusion of pooled plasma or whole blood containing incompatible isoagglutinins may not be entirely a benign procedure. Unless these isoagglutinins are present in low titer, some destruction of recipient cells probably always occurs, which may be manifested solely by an elevation of serum bilirubin. The repeated administration of large amounts of group-O blood or pooled plasma to individuals whose blood groups are other than group-O, appears to result in marked and progressive destruction of the recipient's cells. This phenomenon is usually not accompanied by clinical symptoms such as are ordinarily associated with a transfusion reaction; therefore, except for the development of a variable degree of icterus and anemia, or failure of the anemia to respond to transfusion therapy as anticipated, the hemolytic process may be overlooked. That the destruction of red cells in such cases may be extensive has been illustrated by two cases with severe burns, in whom practically the entire red cell population had been replaced by group-O cells. Another example of this type, observed by the authors, was a patient with leptospirosis icterohemorrhagicae, in whom complete replacement of the recipient group-A cells with group-O cells could be demonstrated following multiple transfusions of pooled plasma and group-O blood.

The degree of hyperbilirubinemia following transfusions of incompatible plasma, as well as the promptness and constancy with which it was observed, was unexpectedly great in proportion to the development of hemoglobinemia. This evidence suggests that the major portion of blood cells destroyed by incompatible plasma is not hemolyzed in the free circulation, but in a site that is to some extent segregated, and in close proximity to the bilirubin-forming tissues. A similar situation may exist in certain naturally occurring hemolytic syndromes, for example congenital hemolytic jaundice, in cases of which hemoglobinemia is ordinarily absent, and in which hemolysis of the congenitally defective erythrocytes is presumed to take place chiefly in the spleen. Hemolytic syndromes attributable to hemolytic systems demonstrable *in vitro,* on the other hand, such as paroxysmal nocturnal hemoglobinuria and paroxysmal hemoglobinuria due to chilling, are characterized by hemoglobinemia, which is unaccompanied by significant hyperbilirubinemia. It is suggested that, in hemolytic diseases of the latter type, the erythrocytes are destroyed by the activated hemolysin intravascularly, as in the test tube, hemoglobin being liberated directly into the free circulation.

Dameshek and Schwartz (13) were able to produce hemolytic anemia in guinea pigs by injecting serum obtained from rabbits immunized against guinea pig erythrocytes. In large doses this serum, which contained agglutinins as well as hemolysins, produced marked hemoglobinuria and death; repeated small doses caused subacute anemia of moderate degree, without hemoglobinuria. With both schedules of dosage there resulted an increase in osmotic erythrocyte fragility. Evidence that corresponding effects may be produced in humans, following transfusion with incompatible plasma, is contained in the results of osmotic fragility studies reported in this communication. Thus, of 4 cases of blood groups A and AB, who received massive quantities of pooled human plasma in the treatment of severe burns, all exhibited a progressive increase of osmotic erythrocyte fragility. It is to be noted that

Shen and Ham (14) have described changes in erythrocyte osmotic fragility in patients with severe burns. However, it is considered that the fragility changes described in the cases studied in the present series are probably not related to a thermal effect, for the patients' red cells were almost totally involved, the fragility changes were progressive over a period of several days, and did not occur when the patients' blood was group-O. Moreover, fragility changes of an identical character were observed following single transfusions of blood containing a high concentration of incompatible isoagglutinins, and multiple transfusions with low-titer agglutinins, in patients who had sustained no burn.

The mechanism of the increased osmotic fragility exhibited by recipients of incompatible plasma is unknown. The salient features of the phenomenon, however, are evidently related to the presence of a specific immunologic property of the incompatible plasma, and not due to a complication of disease or injury, or to some fortuitous, non-specific effect of the transfusion. It is uncertain whether, in these cases, the increased susceptibility of the erythrocytes to hemolysis in hypotonic solutions of sodium chloride is a factor contributing to the hemolytic syndrome, or whether it is merely an index of red cell damage.

With regard to the practical aspects of transfusion therapy, no evidence has been obtained which suggests that the emergency use of group-O blood, containing a low titer of isoagglutinins, may be attended with undesirable consequences. It is important, however, that stored group-O blood, before it is designated for this purpose, should be examined for the concentration of isoagglutinins contained in the plasma, as was done by the Army Blood Bank in the latter phases of the European campaign, high-titer blood being reserved for group-O recipients. On the other hand, in cases requiring *repeated* transfusions, blood of a homologous group should be administered, unless the method of Witbsky (15), designed to neutralize the isoagglutinin potency by absorption with purified A and B substances, proves entirely satisfactory and becomes generally available. Further evidence is required before the limitations of plasma transfusion can be adequately defined. A significant titer of isoagglutinins is frequently demonstrable in commercial brands of dried human pooled plasma, and observations suggest that, when this material is employed in massive dosage or administered repeatedly to patients whose blood groups are other than group-O, rapid blood destruction may result.

SUMMARY AND CONCLUSIONS

1. A study has been made of certain complications attending the transfusion of group-O blood and pooled plasma containing incompatible isoagglutinins. The investigation was conducted in a U. S. Army General Hospital and Evacuation Hospital, during the course of the European campaign.

2. Febrile hemolytic reactions, accompanied by chills, were rarely observed following either single or multiple transfusions of group-O blood into recipients whose blood groups were A, B or AB. Three reactions, accompanied by hemoglobinemia and hyperbilirubinemia, were observed in the course of 265 consecutive transfusions of this type, an incidence of 1.1 per cent. The isogglutinin titer of the blood implicated in these reactions in each case exceeded 1:500.

3. Studies, whch included serial Ashby counts and, in some instances, blood volume measurements, indicated that asymptomatic blood destruction involving the recipient cells was occasionally produced by single, and almost invariably by multiple, transfusions of pooled plasma or group-O blood, when administered to patients of other blood groups.

4. In contrast to the findings after the injection of cell-free hemoglobin solution, a disparity was noted between the degree of hyperbilirubinemia and hemoglobinemia produced by these transfusions. This suggested that most of the hemolysis produced by incompatible plasma does not occur in the free circulation.

5. An increase in the osmotic fragility of the recipient erythrocytes was frequently observed following transfusions of group-O blood and pooled plasma in patients of blood groups other than group-O, the occurrence of this phenomenon being related to the concentration of incompatible isoagglutinins and frequency of their administration.

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RECORDING OF RIGHT HEART PRESSURES IN NORMAL SUBJECTS AND IN PATIENTS WITH CHRONIC PULMONARY DISEASE AND VARIOUS TYPES OF CARDIO–CIRCULATORY DISEASE [1]

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INTRODUCTION

The development of the technique of recording pressures in the right auricle and ventricle by connecting an intra-cardiac catheter [2] to a Hamilton manometer has made possible for the first time in human subjects the direct quantitative study of some aspects of the right heart function and of the pulmonary circulation, in health and in disease. The method has been previously described in brief (1).[3] In the present report tracings obtained from normal subjects and from patients suffering from a variety of pulmonary and circulatory diseases are described in greater detail. The intracardiac pressure curves are analyzed in relation to the different phases of the cardiac cycle, and an attempt is made to give an interpretation of the abnormal curves in terms of the pathological conditions with which they are associated. While reference will occasionally be made to other data routinely obtained in this study, including cardiac output, blood volume, x-rays and electrocardiograms, complete presentation of these is beyond the scope of this paper.

METHODS

A specially designed ureteral type of catheter was inserted into an antecubital vein, and the catheter tip directed into the right auricle or ventricle under fluoroscopic visualization according to the technique described earlier (2 to 4). In the majority of instances a catheter of single lumen was used. A double-lumen catheter, recently designed (5), was employed for simultaneous study of the pressure cycle in both auricle and ventricle in a few selected cases, which are discussed in the text. With proper precaution it has proved feasible to make determinations in a wide variety of patients with safety and apparent accuracy. Due attention to the details of the technique (*i.e.* avoidance of pain, rapid execution of the various procedures, careful selection of the number of determinations to be made, in the light of the patient's clinical status) has resulted in the absence of any significant complication attributable to the method of study. However, provocation of ectopic ventricular beats by the catheter in the ventricle, probably due to contact of the catheter tip with the inter-ventricular septum, has been sufficiently frequent, especially in cardiac patients, to indicate caution in the matter of catheter position. Observation of the continuous electrocardiogram by one of the operators may detect this at once. Thus far it has always been possible to manipulate the catheter in such fashion as to bring about prompt cessation of the abnormal stimuli. No induced arrhythmias have been observed when the tip of the catheter was located in the auricle.

The specifications of the cardiac manometer were essentially those given by Hamilton (6), except that the lead tubing was about 1.5 meters long. The natural frequency was usually 25 to 50 cycles per second, with the catheter attached, and the sensitivity of the instrument for the camera distance of 2 meters was 0.5 to 1.0 mm. per mm. Hg.

In the majority of studies the record also included a simultaneous registration of the electrocardiogram with a string galvanometer, the respiratory cycle through a modified Marey pneumograph, and the femoral arterial pressure, for which a second Hamilton manometer of higher frequency and lower sensitivity was employed.

The determinations in all but a few cases were carried out with the patient recumbent and in the post-absorptive state after a night of bedrest.

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TABLE I

Peripheral venous, right heart, and arterial pressures

Patient number	Age	Sex	Peripheral venous pressure (mm. Hg)	Right auricular mean pressure (mm. Hg)	Right ventricular pressures P_s (mm. Hg)	P_{d1} (mm. Hg)	P_{d2} (mm. Hg)	P_p (mm. Hg)	Femoral artery pressures P_s (mm. Hg)	P_d (mm. Hg)	P_m (mm. Hg)	Diagnoses and remarks**
												Normal subjects
1a	44	M			26	−1	4	22	134	62	88	Normal adult.
1b			5*		24	−2	2	22	138	66	89	Same, 1 week later.
2	56	F			22	−1	1	21	120	50	74	Convalescent from upper respiratory infection. Secondary anemia.
3	22	F	3*		18	−0.5	1	17	105	56	74	Convalescent from pancreatitis. Secondary anemia.
4	39	F	3.5*	1	23	−1	1.5	21.5	123	65	90	Normal adult.
5	43	M			30	2	4.5	25.5	136	80	106	Chronic alcoholism.
6	26	F			30	0.5	3.5	26.5	125	71	92	Normal adult.
7	31	F			25	−1	4	21	127	65	87	Convalescent from upper respiratory infection. Hypometabolism.
8	26	F			28.5	−3	3	25.5	117	62	84	Normal adult.
9	39	M		0.5					123	63	83	Normal adult.
10	43	M	1	2					100	50	63	Normal adult.
11	22	M		−2	23.5	−7	1	22.5	139	86	106	Normal adult. Head and trunk elevated 25°.
12	53	M		1.5					123	67	87	Normal adult.
13a					22	−5.5	−0.5	22.5	138	77	99	Convalescent from upper respiratory infection. Chronic bronchitis. Chronic alcoholism.
13b					24.5	−5.5	4	20.5	137	79	99	Chronic alcoholism. Four months later.
14	31	M	4	−2					133	76	97	Chronic bronchitis. Head and trunk elevated 25°.
15	30	F		1.5	28	−6	3.5	24.5	112	54	75	Normal adult. Head and trunk elevated 25°.
16	22	M		−0.5	27.5	2	3.5	24	149	77	99	Normal adult. Head and trunk elevated 25°.
17	43	M	7	−1					138	75	98	Normal adult.
												Pulmonary emphysema, pulmonary fibrosis, or both, without clinical evidence of cardiac failure
18	44	M	1*	−1	39	−3.5	1.5	37.5	137	82	103	Chronic obstructive pulmonary emphysema.
19	58	M	2*	−1	40	−2	6	34	146	77	102	Chronic obstructive pulmonary emphysema.
20	40	F		−1.5	16	0	4	16	111	56	71	Chronic obstructive pulmonary emphysema.
21	62	M		−2	22.5	−4	4	18.5	106	64	84	Chronic obstructive pulmonary emphysema.
22	60	M	0*	1.5	49	−3.5	4	45	120	63	87	Chronic obstructive pulmonary emphysema.
23	55	M	−2*	−3	45	−4	2	41	156	69	99	Chronic obstructive pulmonary emphysema.
24	59	M	1*	−3.5	28	0.5	3	26	125	69	85	Chronic obstructive pulmonary emphysema.
25	52	M	3.5*	−1	46.5	−2.5	6.5	43.5	128	63	90	Chronic obstructive pulmonary emphysema. Generalized arteriosclerosis.
26a	48	M	6	3	41.5	−1.5	4.5	35	119	71	91	Silicosis. Chronic pulmonary emphysema.
26b			2.5*	1.5	38	−2.5	1	33.5	119	73	99	Silicosis. Chronic pulmonary emphysema.
27	32	M		−1	30.5	0	2	29.5	142	74	86	Nodular pulmonary tuberculosis. Chronic pulmonary emphysema.
28	33	M	0*	1.5	45	−8	2.5	43	114	66	92	Arrested apical tuberculosis. Chronic pulmonary emphysema.
29	53	M	6*	−2	30	−3	3.5	27.5	126	69	92	Same, 5 weeks later.
30	52	M	0*	−5	37	−2	4	33.5	132	71	77	Chronic pulmonary tuberculosis; large cavity; diffuse fibrosis. Probable chronic emphysema.
31	47	M		−3.5	34.5	−6	0	30.5	109	57	93	Chronic pulmonary tuberculosis; bilateral cavities; bilateral re-expanded pneumothorax.
32	42	M	−1*	−1.5	38	−4.5	5.5	38	130	75	97	Bronchiectasis; chronic pulmonary emphysema and fibrosis.
33	41	M	1.5*	−2.5	57.5	−3.5	0.5	52	123	81	84	Bronchial asthma; chronic pulmonary fibrosis; recovering from bronchopneumonia.
34	53	M		0.5	50.5	−4.5	1.5	50	117	66		Diffuse nodular pulmonary fibrosis, cause unknown.
35					55.5	0	5	54	161	84	116	Diffuse nodular pulmonary fibrosis, cause unknown.
36	62	M			45			40	127	70	89	Diffuse nodular pulmonary fibrosis, cause unknown.
												Pulmonary emphysema, pulmonary fibrosis, or both, with clinical evidence of cardiac failure
37	62	M	13	13	35(?)	7	14	21(?)	157	74	105	Convalescent from right heart failure; on digitalis.
												Post-pneumonectomy
38	65	M	9.5*	14	77	7	9	68	133	64	89	Chr. pulm. emphysema and fibrosis. EH, CS, MF, NSR, IIID. Partial recovery from r. heart failure.
39a	51	M	5	8	81/40	8/11	9/12	69/31	158	88	111	Chr. pulm. emphysema and fibrosis. EII, NSR, IIID. Partial recovery from right heart failure.
39b				2					146	79	103	Silicosis. Arteriosclerosis. EII, NSR. Compensated. Same, 6 months later.
39c				7					174	81	107	Right heart failure. Same, 1 month later. Right heart failure; digitalis intoxication.
40	23	F		−2.5	26	−6	−4	30	125	81	95	Pneumonectomy 6 weeks previously for large air cyst of right lung.
41	67	M		−0.5	25	−3	2	23	156	80	109	Pneumonectomy 3 years previously for bronchial carcinoma of left lung.
												Fibrothorax
42	44	M	2*	−1	26	0	26	26	119	67	86	L. fibrothorax. Apical tuberculosis with cavity and chronic emphysema and fibrosis of r. lung.
43	32	M			26.5	−1.5	28	28	113	68	83	R. fibrothorax. Pulmonary tuberculosis, IIIB.

TABLE 1—Continued

Patient number	Age	Sex	Peripheral venous pressure	Right auricular mean pressure	Right ventricular pressures P_s	P_dI	P_dII	P_dIII	P_p	Femoral artery P_s	P_3	P_4	P_m	Diagnoses and remarks**
														Kyphoscoliosis
	55	M	9*	0	36	-1			35		155	97	119	Severe kyphoscoliosis. Chr. emphysema and fibrosis. Gen'l arteriosclerosis. Hypertension.
														Pneumothorax
	66	M		-0.5	19	-2.5	1		18		118	62	82	Pulmonary tuberculosis, IIB. Right pneumothorax induced 5 days previously.
		M		1	25	-2	2		23					Pulmonary tuberculosis, IIIB. Left pneumothorax induced 23 days previously.
		M									148	77	100	Pulmonary tuberculosis, IIIB. Right pneumothorax induced 10 days previously.
		M			22.5	-1	.5		19		109	67	81	Pulmonary tuberculosis, IIIB. Left pneumothorax induced 12 days previously.
		M		-1.5	27	-7.5	-2		29		131	75	94	Pulm. tbc., arrested. Chr. emphys. and fibr. Spontaneous pneumo. 2 days previously. On oxygen.
														Rheumatic heart disease
	22	F	25*	0.5	13.5	-5.5	2.5		41		113	56	76	EH, MS, MI, NSR (occasional nodal rhythm, wandering pacemaker), IIC.
	55	M	5.5*	29	103	10	29		74		210	96	137	EH, MS, MI, AI, TI, AF, IVE. Right and left heart failure. Hypertension.
				20.5	57	0	-4		53		165	61	98	Same, 1 month later, after treatment. TI and heart failure signs absent.
	63	M	21	17.5	116.5	8.5	21.5		95		181	76	129	EH, MS, MI, TI, AF, IVE. Right and left heart failure. Hypertension.
	12	M		16							163	72	103	EH, MS, MI, AS, AI, TI, AF, IVE. Right and left heart failure.
	18	M	11.5	27	95.5	4	16.5		79		157	89	103	EH, MS, MI, AS, TI, AF, IVE. Right and left heart failure.
	45	M	23.5	16	85.5	6.5	16.5		69		125	72	91	EH, MS, MI, AI, TI, AF, IVE. Right and left heart failure.
	57	M		16										EH, MS, MI, TS(?), TI, AI, AF, IIID. Partial recovery from right and left heart failure.
		M												EH, MS, MI, TS, TI, AI, AF, IIIE. Partial recovery from right and left heart failure.
														Arteriosclerotic heart disease
	56	M	10.5*	13	65	6	12		53		130	70	87	EH, CS, MF, bundle branch block, AF, subsiding digitalis intoxication, IIID.
														Syphilitic heart disease
	69	M	9*	13	72	2	13		59		149	37	79	EH, AI, aneurysm of aorta, NSR, IVE. Right and left heart failure. Gaucher's disease.
														Heart disease of unknown etiology
	48	F	15*	0.5	23	-3.5	0		23		151	89	112	EH, NSR. Not in cardiac failure.
			9.5*	2.5	60	7.5	13		47.5		190	129	155	Same, 4 months later. Right and left heart failure. Hypertension.
	37	M	5*	1	31	3.5	2.5		27.5		100	59	83	Beri-beri (?), interstitial myocarditis (?). Not in cardiac failure.
														Congenital heart disease
	26	M	15*	18.5	77.5	-1	0		68.5		119	82	99	Intermittent cyanotic group; EH, probable inter-auricular septal defect, AF, RVF, IIC.
	49	M	15*	2.5							118	53	72	EH, inter-vent. sept. defect, NSR, no failure. Laennec's cirrhosis; lymph node tbc., syphilis.
														Constrictive pericarditis
	40	M	15*	18	31	10	20		11		98	66	77	Chr. constr. pericard., adhesive, due to wound of thorax; pleural effusion; c.p.c. liver and spleen
														Arterial hypertension
	45	M			25.5	0			22.5		158	90	114	Essential hypertension. After sympathectomy.
	49	M			21.5	1.5	5		19.5		190	98	132	Essential hypertension. After sympathectomy.
	35	M			30.5	-5.5	2.5		28		206	111	150	Essential hypertension. After sympathectomy.
	65	M	5*		21	-1	2		22		159	83	111	Hypertension, etiology undetermined. Convalescent from pneumonia. Secondary anemia.
	35	M			32		-1.5		20.5		173	101	128	Hypertension, etiology undetermined. Chronic alcoholism.
														Shock
	35	M			22	-5.5	-0.5		22.5		138	77	99	Control. See Normal Subjects above.
		M			15.5	-0.5			11		16	31	36	Immediately after 20 per cent of blood volume removed in 20 minutes.
	46	M			9.5	-3.5			13		103	16	65	Chronic anemia due to bleeding peptic ulcer. Recent large hemorrhage; mild shock.

* Venous pressure measured with saline manometer and converted to mm. Hg.
** Cardiac diagnoses are given in accordance with the "Nomenclature and Criteria for Diagnosis of Diseases of the Heart" of the New York Heart Association, 4th edition, 1941. Key to abbreviations: EH, enlarged heart; MS, mitral stenosis; MI, mitral insufficiency; AS, aortic stenosis; AI, aortic insufficiency; TS, tricuspid stenosis; TI, tricuspid insufficiency; CS, coronary sclerosis; MF, myocardial fibrosis; NSR, normal sinus rhythm; AF, auricular fibrillation; RVF, right ventricular failure.

Tracings were routinely measured for pressure values in each heart beat during normal complete respiratory cycles, and the results averaged. Mean pressures were determined by planimetric integration. The data from arterial pressure records include average maximum (systolic), average minimum (diastolic), and mean pressures. For the auricle, only mean pressures are given in the tables, although momentary pressures were sometimes also measured. Because of the frequently oscillatory character of the ventricular pressure curve, the average value for the highest level attained during the ventricular ejection period was arbitrarily chosen to represent the systolic pressure, and is designated P_s. The pressures at two moments during ventricular diastole were measured: (a) at the lowest point reached following closure of the pulmonic valve, and (b) at the end of auricular systole, or when the latter was not apparent in the ventricular tracings, just before the onset of the ventricular systolic rise. These are designated P_{d1} and P_{d2}, respectively (Figure 2C). The significance of the earlier diastolic pressure is discussed below. P_{d2} was chosen as a measure of the initial, or filling pressure, of the ventricle. The difference between the maximal systolic pressure and the pressure at the end of diastole (P_s minus P_{d2}) is designated as the pulse pressure (P_p). Auricular mean pressure proved to be an accurate index of mean ventricular diastolic pressure, as substantiated by withdrawing the catheter from ventricle to auricle during uninterrupted or momentarily interrupted recording (Figure 1B).

All pressures are recorded in mm. Hg above or below atmospheric pressure taken as zero. To eliminate hydrostatic factors it is necessary to choose some horizontal plane as a level of reference. For intra-cardiac pressures it appears that this plane should pass somewhere through the heart itself and at the same time bear a reasonably constant relationship to an external landmark such as the angle of Louis. As an approximation, therefore, the depth of the center of the heart below the angle of Louis was measured from a lateral x-ray as the vertical distance from the angle of Louis, to the mid-point between the anterior tip of the ventricle and lowermost portion of the auricle, the patient being in the horizontal position. In normal subjects this measurement averaged 5.82 cm., with a total range of ± 0.90 cm. In patients with cardiac enlargement it averaged 5.55 ± 1.50 cm. In cases of emphysema it averaged 6.86 ± 0.80 cm. For the sake of simplicity, however, it has been decided to take the conventional venous pressure reference level of 5 cm. below the angle of Louis as the one from which to reckon *both* auricular and ventricular pressures when the patient is recumbent. This level, then, could differ from zero plane exactly through the center of the heart by as much as 2 or 3 mm. Hg. The true reference point, moreover, will vary in any given patient with the phases of the respiratory and cardiac cycles. Hence no value of pressure except ventricular pulse pressure can be considered to have a final precision greater than ± 2 or 3 mm. Hg when patient to patient comparison is made. For studying changes in a given patient, however, the choice of a reference level is of little importance.

While the data from the present study have yielded useful information, it is realized that there may have been errors, both of technique and interpretation. In general, the sources of error are twofold: (a) reference to an incorrect zero may distort pressure values and lead to a false appraisal of physiologic states; (b) failure to recognize artefact may result in erroneous computation of pressure values or incorrect interpretation of physiologic events. With respect to the latter, every effort has been made to profit by the experience of others, and to detect new artefacts introduced by the present technique. Such obvious sources of invalid curves as motion by the patient, and extraneous impacts upon equipment are usually recognizable and avoidable. There remain, however, certain oscillatory phenomena which are subject to controversy, in particular those seen at the crest of systole and early in diastole, which may be due entirely to motion of the catheter or may be caused, in part at least, by oscillations of the blood mass.

RESULTS

The data in Table I are from 77 catheterizations in 70 subjects. In 12 instances, right auricular pressure tracings alone were taken; in 19, right ventricular tracings alone were taken; in 46, tracings were made from both chambers. Five patients were studied twice; one patient was studied 3 times.

It is obvious that any numerical description can give only a partial concept of the tracings, and it is therefore esential to consider not only the amplitude of pressure curves, but also their contours. The possibility of artefact must be considered whenever an unusual finding is to be evaluated. Correlation must be made with the known sequence of physiological events in the cardiac cycle, and final interpretation must be based on consideration of all the known clinical facts.

Throughout this report only the *right* heart pressures are discussed. The terms, auricle, auricular, ventricle and ventricular will therefore always refer to the *right* side of the heart.

I. Normal subjects

A. Right auricle. In resting recumbent normal subjects the mean auricular pressure has varied from − 2 to + 2 mm. Hg, with respect to atmospheric pressure. The several components of the cardiac cycle are, in good curves, readily recognizable, and correspond closely to those described by Wiggers (7). The magnitude of pressure variation within the cycle is small, being of the order
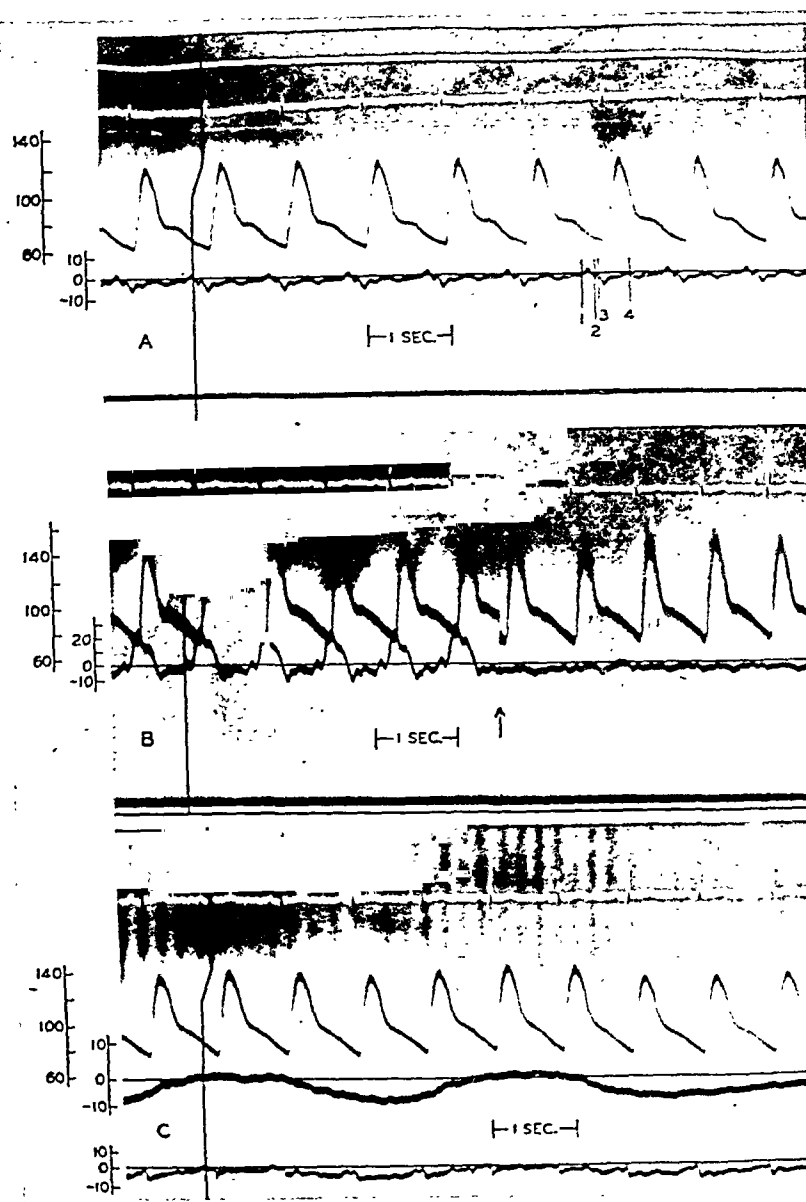
FIG. 1. A. RECORD FROM NORMAL MALE, PATIENT No. 9

In this and subsequent tracings, electrocardiogram and pneumogram, where shown, are at top of film. Descent of white line indicates inspiration; ascent, expiration. Upper black curve is femoral arterial pressure; next lower is that of right auricle (as in this case) or ventricle. Time in subdivisions of 0.04 and 0.20 seconds is registered on baseline. Scales in mm. Hg, drawn at left of figure, represent static calibration against mercury for each case. Parallax between electrocardiogram and pressure curves is indicated by vertical line drawn through beginning of ventricular complex of electrocardiogram. It is usually equivalent to 0.13 second. Events of auricular cycle are 1, rise of pressure due to auricular systole; 2, sharp rise of pressure due to closure of tricuspid valve; 3, fall of pressure due to descent of base associated with ventricular ejection; and 4, fall of pressure associated with opening of tricuspid valve and onset of ventricular filling.

B. RECORD FROM PATIENT No. 16, SHOWING NORMAL VENTRICULAR AND AURICULAR PRESSURES

Arrow indicates brief closure of camera while catheter tip was withdrawn from ventricle to auricle. Note correspondence between auricular and ventricular diastolic levels.

C. FEMORAL ARTERIAL, INTRAPLEURAL AND AURICULAR PRESSURES IN PATIENT No. 45

Auricular pressure varies in same direction as intrapleural, but to lesser extent. Precise effect on general pressure is also apparent. At maximum expiration, intrapleural pressure appears positive. Note particularly the pressure; presumably due to error in choosing 5 cm. below angle of Louis as reference level for zero. The pressure is

of 4 to 8 mm. Hg. Figure 1A is an unusually satisfactory example of a normal auricular pressure record. Auricular systole appears as a monophasic positive wave, the onset of which is labelled 1 in the figure. Associated with the onset of ventricular contraction is a small initial pressure rise, 2, followed by a rather sharp fall, 3. The initial rise, not present in all tracings, has been ascribed by Wiggers (7) to impact of the closing A-V valves on the intra-auricular blood mass, or possibly to slight regurgitation before closure; the pressure fall is attributed to descent of the base of the heart during ventricular ejection. During the remainder of ventricular systole the intra-auricular pressure gradually rises, until the A-V valves reopen. At this moment, 4, the pressure falls fairly abruptly as auricle and ventricle become a common chamber, after which it again rises gradually until the next auricular systole. Occasionally, just before this auricular emptying phase, a small vibration may be noted simultaneous with the closure of the pulmonic valves.

Even during very quiet breathing a periodic variation of right auricular pressure is usually detectable; this is barely discernible in Figure 1A. To what extent the change in intra-thoracic pressure is reflected in the heart cannot be determined for any particular individual unless intra-pleural pressure is simultaneously recorded. Such a record, obtained by a method recently described (8), from patient No. 45, studied prior to a refill of a pneumothorax induced 5 days previously, is shown in Figure 1C. While this subject was not wholly normal, there is no reason to believe that
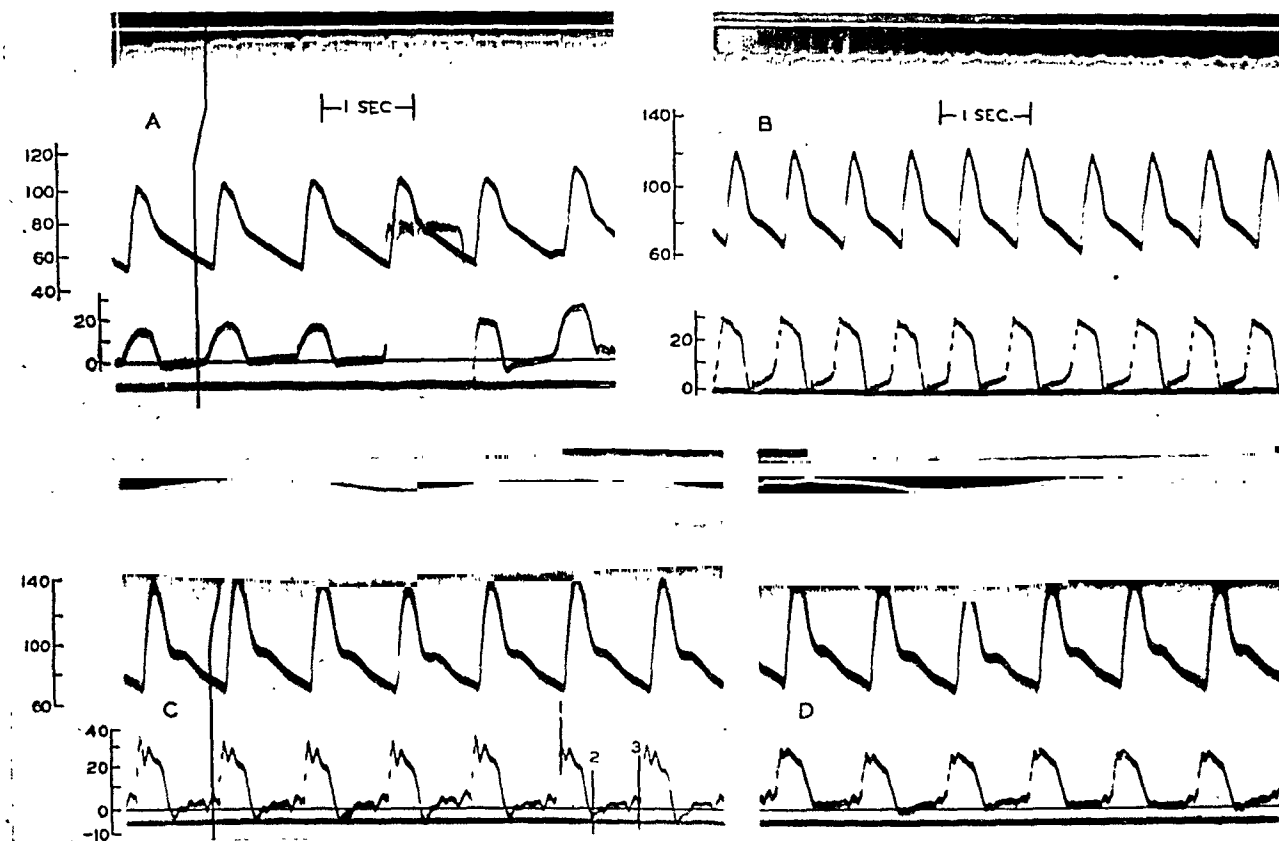
FIG. 2. REPRESENTATIVE RECORDS FROM NORMAL RIGHT VENTRICLE

The sudden interruption in A was caused by flushing citrated fluid through the manometer to insure continued patency of catheter. There is high frequency vibration of extraneous origin in B. In C, lines marked 1, 2, and 3, indicate time of cardiac cycle at which P_s, P_{d1}, and P_{d2}, respectively, were measured. These 3 points were measured in each cardiac cycle comprising one or more complete respiratory cycles, and results averaged. Records C and D were obtained from the same patient (No. 17), the latter after catheter position in ventricle had been slightly changed. Note decrease in amplitude of oscillations while general form of pulse is unchanged. Data in Table I for this patient were measured from D. See text for details.

his circulation was abnormal. The depth of respiration is somewhat greater than in Figure 1A, due to slight emotional disturbance. The ratio of the change of auricular pressure during the respiratory cycle to the change of intra-pleural pressure is about 5 : 8 mm. Hg. or 60 per cent.

B. Right ventricle. As shown in Table I the maximum systolic pressure in normal subjects has ranged between 18 and 30 mm. Hg above atmospheric pressure, averaging 25 mm. Hg for this series. The diastolic pressures have ranged from between − 7 and + 2 mm. Hg at the lowest point early in diastole (P_{d1}), to between − 0.5 and + 4.5 mm. Hg at the end of diastole (P_{d2}). It is of interest that the right ventricular pulse pressure range (P_p) was, with one exception, within the narrow limits of 20.5 to 26.5 mm. Hg, and averaged 22.5 mm. Hg.

Respiratory rate and intra-thoracic pressure variations affect the ventricular pressure curve, as is demonstrated in Figure 2D. In the majority of ventricular tracings, auricular systole is manifest as a small wave occurring just before the onset of ventricular systole (Figures 1B, 2D). Three main types of ventricular systolic contour have been observed. Figure 2A from patient No. 3 is that of an infrequently obtained curve which is smoothly rounded during ejection, falls to a minimum following closure of the semi-lunar valves, and then rises in a gradual and uninterrupted manner during diastole. Equally infrequent is the curve shown in Figure 2B (patient No. 6), which reaches a maximum early in systole. has a rather flattened slope during the rest of the ejection period, and in its diastolic portion resembles the first type. The most frequently observed curves are of the type shown in Figure 1B from patient No. 16, and Figures 2C and 2D from patient No. 17. The summit is marked by one or more low frequency oscillations which may occur at any time during maximal ejection; a small, brief dip below the general diastolic level is usually seen following closure of the pulmonic valve; this dip may initiate a series of damped, low frequency oscillations which last during a variable portion of diastole.

II. Lesions predisposing to pulmonary hypertension and right ventricular strain

A. In the absence of right ventricular failure

1. Chronic pulmonary disease. This group consists of patients with chronic pulmonary emphysema, with chronic pulmonary fibrosis, or with both. The diagnosis of emphysema was based on the measurement of the residual air, the ratio of

TABLE II

Hematocrit, lung volume data, and right ventricular pulse pressures from patients with chronic pulmonary emphysema, fibrosis, or both

Patient number	Hematocrit	Total lung volume		Residual air		$\dfrac{\text{Residual air}}{\text{Total lung vol.}} \times 100$		Right ventricular pulse pressure
		Predicted	Observed	Predicted	Observed	Predicted	Observed	
	per cent	ml.	ml.	ml.	ml.	per cent	per cent	mm. Hg
18	51	4820	5960	1175	3510		59	37.5
19	49	5030	5980	1225	3840		64.5	34
20	42	5660	6860	1380	3470		50.5	16
21	37	3580	3830	880	2280		59.5	18.5
22	42	4960	5000	1210	3160		63.5	45
23	46	5180	5460	1265	4010	24.5	73.5	41
24	43	5660	5910	1385	2710		46	26
25	44	5040	4320	1230	2170		50	43.5
26a	53	4880	5640	1195	3620		64	35
26b	45	4880	5160	1195	3520		64.5	33.5
27	40	6520	7230	1595	2870		40	29.5
29	50	6300	4950	1230	2660	19.5	53.5	27.5
30	47	4690	4250	1120	2670		63	33.5
32	40	5120	2260	1250	1200		53	55
33	47	5660	3120	1385	1860		60	52
34	51	5520	4900	1350	2260		46.5	50
35	48	6020	3610	1470	1620	24.5	45	53
36	49	6160	4450	1510	2010		45	50
37	54	5180	3410	1270	2200		64.5	33
38a	60	5180	3060	1270	1240		39	45
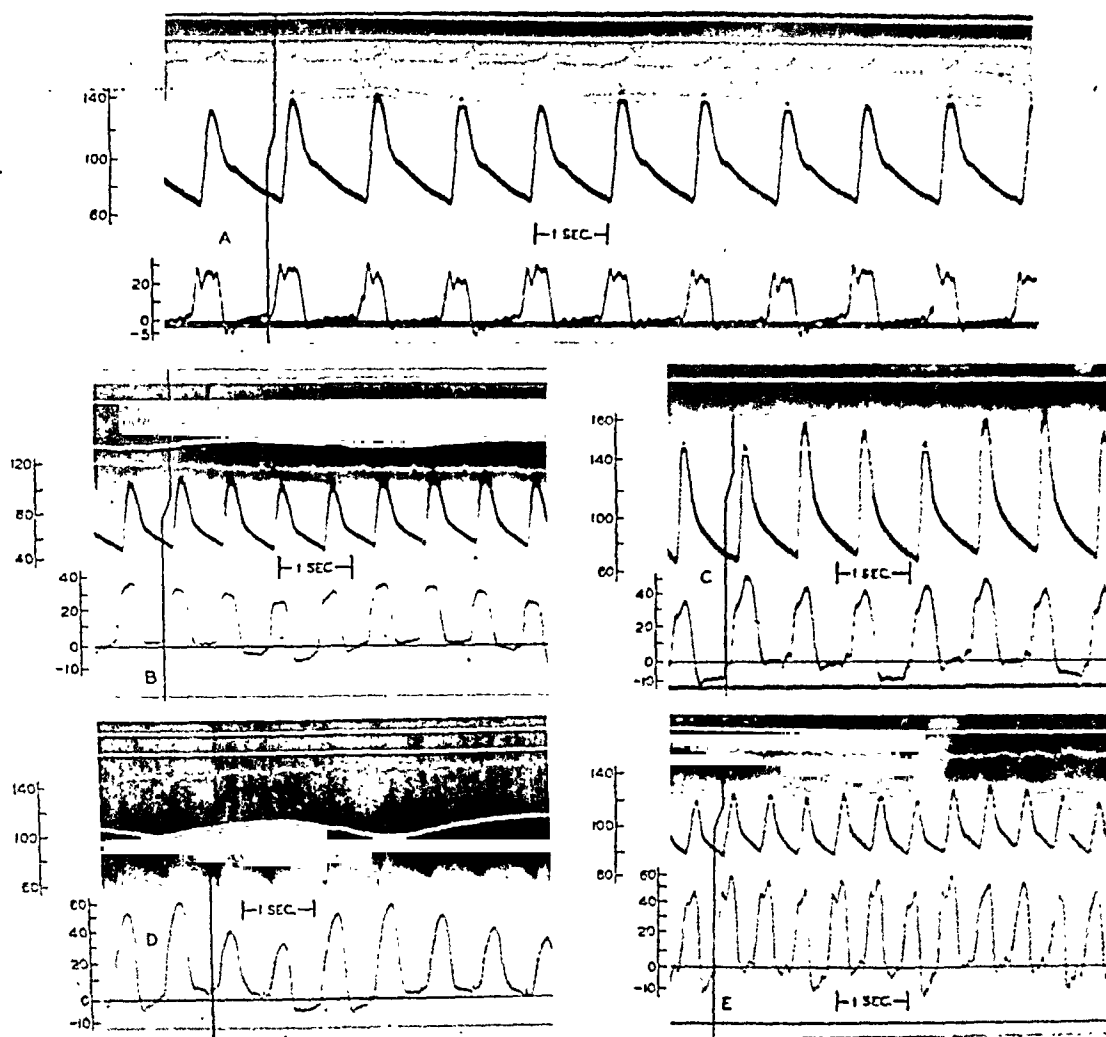
FIG. 3. VENTRICULAR PRESSURE RECORDS IN CHRONIC PULMONARY DISEASE

These were recorded in patients No. 27, 31, 23, 25, and 33, respectively, and show gradations from normal values in *A* to marked systolic elevation, averaging 57.5 mm. Hg, in *E*. Average diastolic pressures are all normal. Note pronounced respiratory variation in all but *A*, reflecting marked pleural pressure changes which occur during the respiratory cycle in chronic pulmonary emphysema (11).

residual air to total lung volume (9), and on the chest x-ray. The diagnosis of pulmonary fibrosis was made on the basis of reduced total lung volume and the x-ray picture. Table II presents the data from the lung volume studies, and compares the observed results with the normal values predicted on the basis of age, sex, and body size (10). The hematocrit indicates the degree of secondary polycythemia. At the time of these studies, none of the 19 patients of this group presented clinical evidence of right ventricular failure or had elevation of auricular and diastolic ventricular pressures. In five cases (Nos. 20, 21, 24, 27, and 29) the pressure measurements were either wholly normal or deviated insignificantly from

the normal range. All others showed definite elevation of ventricular systolic and pulse pressures, the systolic range being from 34.5 to 57.5 mm. Hg and the pulse pressure ranging from 30.5 to 54 mm. Hg. The variations observed in this group are illustrated in Figure 3.

2. Post-pneumonectomy. The first patient, a young female (No. 40, Figure 4A), was studied 6 weeks after the right lung had been removed because of a large air cyst. Measurements of the remaining lung indicated some distention associated with moderate displacement of the mediastinum; otherwise lung function was normal. Ventricular systolic pressure was normal and the pulse pressure was only 2 mm. Hg above the upper limit
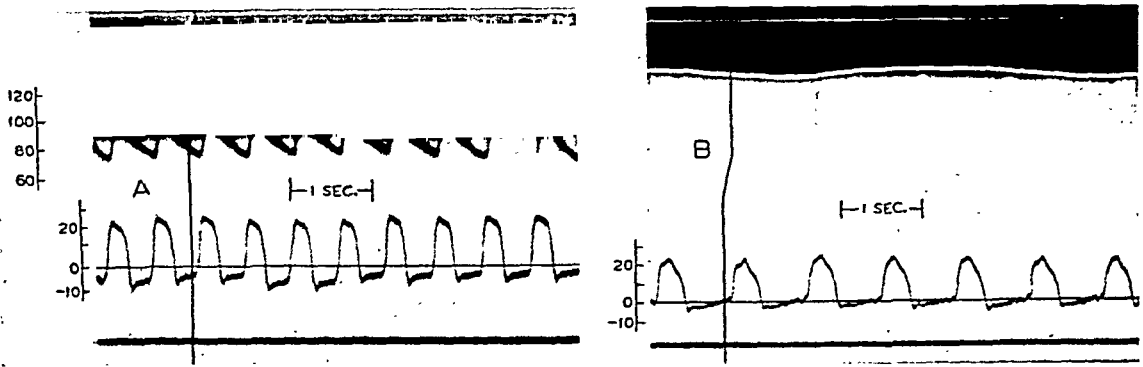
FIG. 4. A. VENTRICULAR TRACING FROM PATIENT No. 40, PNEUMONECTOMY

Low diastolic pressure could be due to abnormally low intrathoracic pressure consistent with observed mediastinal displacement, or due to fact that heart was farther than usual below angle of Louis, or possibly to both.

B. VENTRICULAR PRESSURES FROM PATIENT No. 41, PNEUMONECTOMY

Essentially normal record. See text for details.
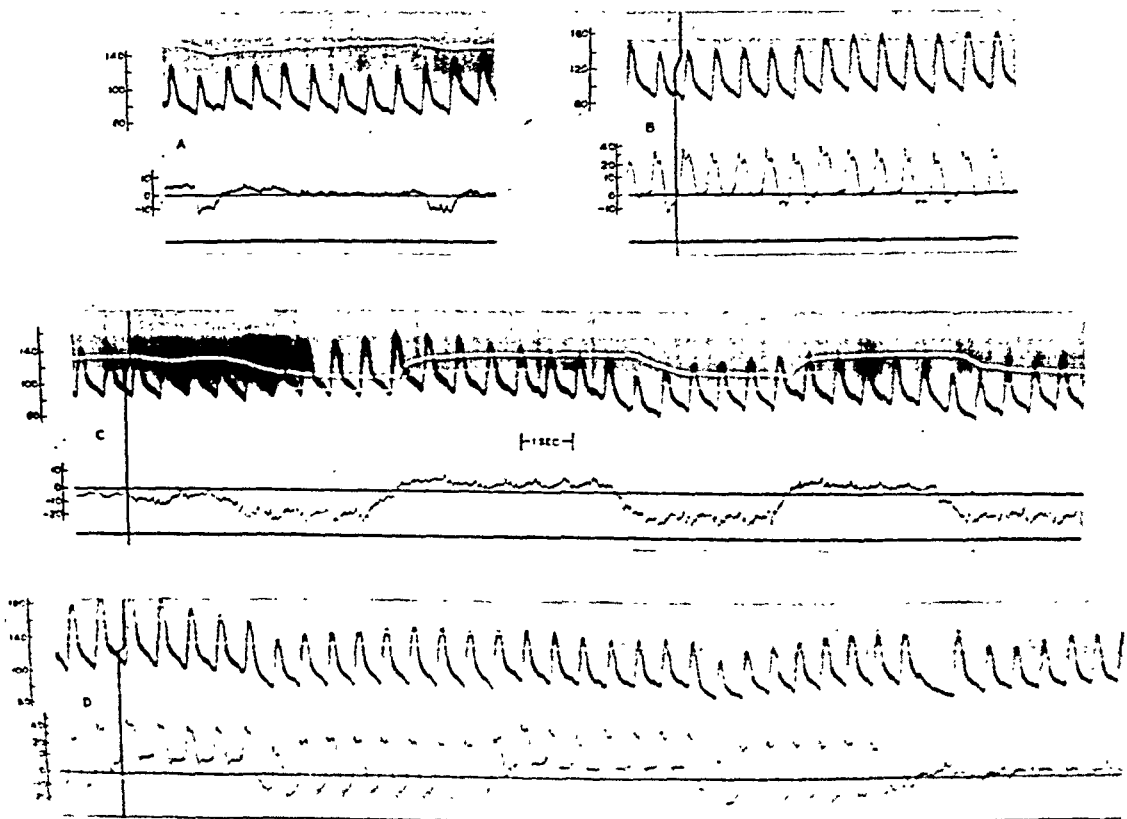
FIG. 5. PATIENT No. 44, KYPHOSCOLIOSIS, WITH EMPHYSEMA AND FIBROSIS

A and B were recorded during quiet breathing; C and D, during deep respiration. Ventricular pulse pressure increase during inspiration in D is distorted by non-linearity of calibration scale. In deep inspiration, near end of D, descent of heart caused catheter to slip back into auricle; nodal premature systole was elicited. Descriptive details in text.

of normal. The second patient, an elderly male (No. 41, Figure 4B), was studied 3 years after the left lung had been removed because of bronchial carcinoma. The function of the remaining lung was normal, and no displacement of the mediastinum was seen on x-ray films. The right ventricular pressures were also normal.

3. *Fibrothorax.* One of the 2 patients, No. 42, had a complete left fibrothorax following re-expansion of an old pneumothorax. Apical tuberculosis with cavitation, and emphysema, were present on the right; there was marked displacement of the mediastinum to the left. Average ventricular systolic and pulse pressures were normal. The tracings displayed sharp and marked variations in level with normal respiration. The ventricular tracings from the second patient, No. 43, were completely normal.

4. *Kyphoscoliosis.* The patient studied, No. 44, had emphysema and fibrosis in addition to a marked chest deformity. The average values for the ventricular systolic and pulse pressures during natural respiration were moderately above normal, being 36 and 35 mm. Hg, respectively. Although the auricular mean pressure was normal, the curve (Figure 5A) showed a sharp and brief fall at the beginning of inspiration, probably reflecting the pleural pressure pattern of severe emphysema (11). This phenomenon was likewise seen in the ventricular record (Figure 5B). During deep and prolonged respiration (Figure 5C), the auricular pressure varied, as a result of the variation in intrathoracic pressure, from about − 18 mm. Hg during inspiration to about + 8 mm. Hg during expiration, a range of about 26 mm. Hg. In the ventricular tracing (Figure 5D) made under similar circumstances, the same extreme range is seen in the diastolic pressure level, and for the same reason. It will be noted, however, that the ventricular pulse pressure increased about 10 mm. Hg during inspiration, strongly suggesting a greater right ventricular stroke volume during this phase of respiration (12).

5. *Pneumothorax.* Of the 5 patients in this group, 4 (No. 45 to 48) had a therapeutic pneumothorax induced 5 to 23 days prior to the study. In these cases, the right heart pressures, recorded immediately before a refill, were entirely within the normal range. The tracing from patient No. 45 has been shown in Figure 1C. The fifth patient (No. 49) had developed a spontaneous pneumothorax 2 days previous to study and had been maintained on 100 per cent oxygen by mask without intervening aspiration. Here, too, the pressures were normal.

6. *Mitral valvulitis with stenosis and insufficiency.* Patient No. 50 was a typical case of rheumatic heart disease with clinical evidence indicating involvement of the mitral valve alone. On auscultation, a loud rumble throughout diastole with a marked presystolic crescendo was heard. There were physical and x-ray signs of slight pulmonary congestion and edema, and fluoroscopy revealed enlargement of the left auricle and right ventricle. No signs of right heart failure were present. Peripheral venous pressure was normal. Ventricular systolic and pulse pressures, 43.5 and 41 mm. Hg respectively, were almost twice the normal value.

Comment

It is interesting to find how little evidence of right ventricular strain there may be in some cases of marked pulmonary emphysema or fibrosis. At least 2 of the patients with severe emphysema had normal right ventricular pressures (Table II). In the pneumonectomy cases, the preservation of normal pressure-flow relations could be achieved either by an increase in the number of small blood vessels perfused in the remaining lung, or by a small increase in their diameters. In case the right heart output is diminished, even a lesser increase in the number or in the caliber of the vessels would be required. In the first of the 2 patients (No. 40) the cardiac index of 3.48 L. per minute per sq. m. of body surface was within the normal range; but in the other (No. 41) the cardiac index was 2.28, which is 73 per cent of the average normal value (3). With regard to fibrothorax and kyphoscoliosis, where variable degrees of mediastinal distortion may be added to variable degrees of reduction in the vascular bed, it is again noteworthy that these conditions are compatible with little or no elevation of right ventricular pressure.

The finding of a high ventricular systolic pressure in the presence of severe mitral stenosis is consistent with the classical conception of the effects of this lesion on the pulmonary circulation and the right ventricle.

Because the right heart and pulmonary artery have hitherto been inaccessible to direct study in man, the concept of *cor pulmonale, i.e.* heart disease due to pulmonary disease has been generally less familiar and less understood than the analogous concept of hypertensive heart disease. The mechanical basis, of course, is pulmonary arterial hypertension. The present method makes it possible to detect such hypertension and to express somewhat quantitatively the degree of chronic right ventricular strain, at a time when the peripheral venous and right auricular pressures are normal, and when radiologic and electrocardiographic data may be equivocal or of no clinical value.

B. *In the presence of right ventricular failure*

It was seen in the patients having chronic pulmonary disease without evidence of heart failure, that when the right ventricular pressure exceeded normal levels, it did so only during systole. So long as the right ventricle, pumping against increased pulmonary resistance is capable of delivering a normal stroke volume under the condition of a normal filling pressure, the patient being at rest, it is considered to be fully adapted to its task; diastolic pressure, as well as auricular and peripheral venous pressures, are then not elevated above the normal. However, when the right ventricle "fails," [4] pressure pulse contours from the right heart and peripheral venous system reveal characteristic peculiarities. While all of the points to be mentioned may not be prominent in any given case, in their entirety the records present the following features: (a) ventricular systolic hypertension; (b) abnormal elevation of the general level of the ventricular diastolic pressure; (c) marked rise of the ventricular diastolic pressure above the minimum which occurs early in diastole, giving rise to a sharp early diastolic "dip" between the arched down-curve of isometric relaxation and the plateau-like remainder of diastole; (d) elevation of the mean auricular pressure; (e) accentuation of the drop in auricular pressure at the time of descent of the base resulting from ventricular ejection; (f) exaggeration of the fall

of pressure in the auricle at the time of rapid emptying into the ventricle, corresponding closely in time with the early diastolic dip in the ventricle (items (e) and (f) give to the auricular curve the shape of a "W"; however, as will be shown below, item (e) is absent if tricuspid insufficiency is present); (g) elevation of the peripheral venous pressure; (h) appearance of retrograde transmission into the peripheral venous system of intra-auricular pressure variations, especially of the negative waves; (i) diminution of the normal gradient of pressure between the antecubital veins and the right auricle, often to the point where the two differ by but a few mm. of saline (13, 14).

1. Chronic pulmonary disease. Patient No. 37 was beginning to recover from right heart failure at the time of study. He was able to be out of bed, but edema persisted. The ventricular diastolic and auricular pressures were still elevated. The ventricular systolic pressure was only slightly elevated to 35 mm. Hg, and therefore the pulse pressure was normal. Because some doubt exists regarding the validity of the tracings during the systolic intervals, no further interpretation is justified at present. The auricular, as well as the ventricular diastolic records are reliable, however, and are typical of right heart failure.

Patient No. 38 was also studied after partial recovery from right heart failure. Ventricular catheterization was unsuccessful. The right auricular curves showed elevation of the mean pressure, as well as the "W" contour, and the peripheral venous pressure, measured with the saline manometer, was above normal.

Patient No. 39 exhibited clinical evidence of right heart failure as a result of advanced pulmonary fibrosis due to silicosis. Studies were done on three separate admissions, during the first and last of which he was in obvious failure, while at the time of the second cardiac compensation had returned. Pressure tracings from the 3 studies are shown in Figures 6 and 7. In Figure 6A the ventricular systolic pressure at 77 mm. Hg. and the diastolic pressure, which ranges between 7 and 9 mm. Hg. are both abnormally high. Because of the low frequency oscillation due to artefact, the early diastolic dip cannot be accurately identified. In the auricular curve artefact also obscures finer details, but the elevation of the mean pressure to 8 mm. Hg is characteristic

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[4] "Failure" is used in its clinical connotation to indicate increased diastolic filling pressure and venous congestion. A clear physiologic distinction between "competency" and "incompetency," "compensation" and "decompensation" of the right heart would require consideration of many factors outside the province of this paper.
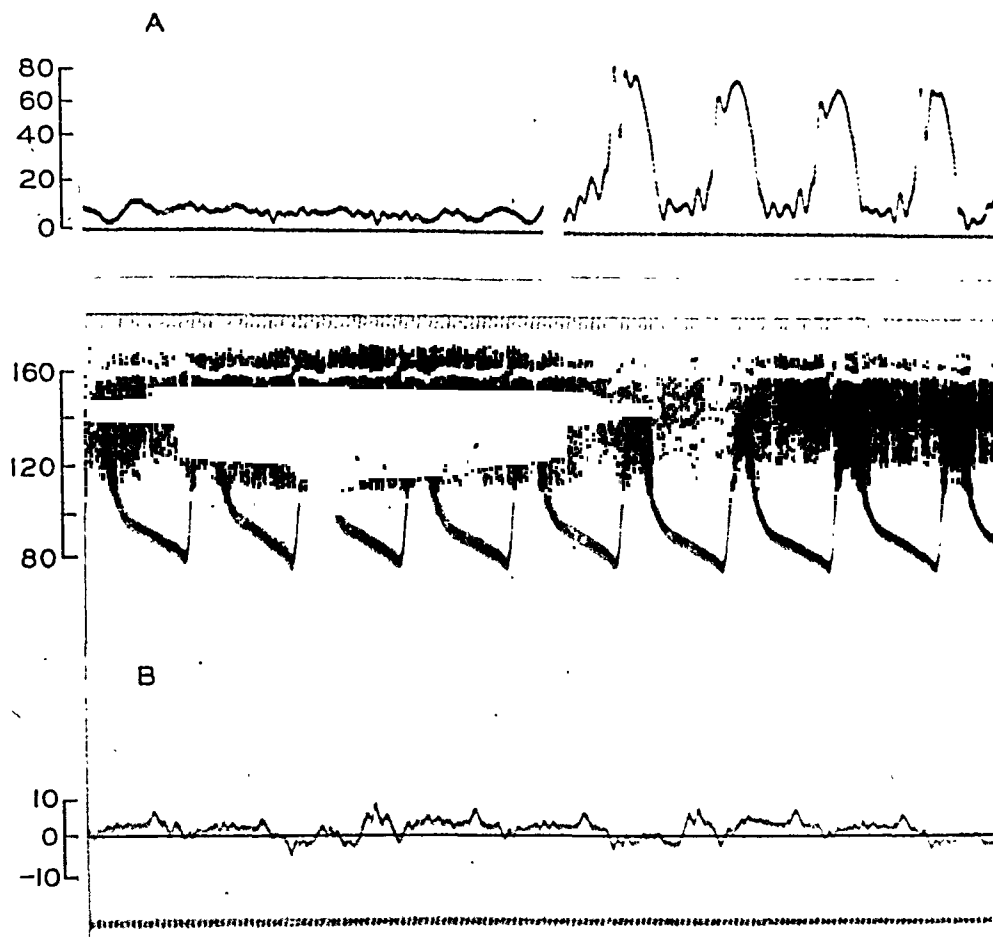
FIG. 6.   A.   FIRST STUDY OF PATIENT No. 39, IN RIGHT HEART FAILURE
Left, auricle; right, ventricle.

B.   SECOND STUDY OF SAME PATIENT, HEART COMPENSATED
Auricular pressure has returned to normal.   See text for details.

Ventricular catheterization was unsuccessful at the second study, but the auricular tracing has returned to a normal level (Figure 6B).

At the time of the third study tracings were taken from both auricle and ventricle, during quiet and forced respiration. The upper 2 sections of Figure 7, right auricle and ventricle respectively, were made during quiet breathing. High ventricular systolic pressure prevails, and the diastolic and auricular pressures are of the same order as in the first study. The bigeminal rhythm, due to ectopic ventricular beats resulting from digitalis toxicity, lends some striking characteristics to the curves and exemplifies the variety of data which this technique may produce for detailed study. Alternation in the systolic heights and pulse pressures of the ventricular beats is referable to the marked difference in filling time preceding the respective contractions. In the lower 2 sections, recorded during deep respiration, the early diastolic dip following the premature ventricular beats, is especially characteristic during inspiration. However, the premature beats come so early in the diastole of the preceding normal (post-extrasystolic) beat, that the ascending limb of the dip, due to rapid refill from the auricle, merges immediately with the upstroke due to the premature systole, and therefore these dips are not typical. (From a hemodynamic standpoint there are no normal beats in this rhythm; the beats of normal sinus origin follow the longer-than-normal compensatory pause, and therefore are associated with stroke volumes greater than would be expected with a regular and uninterrupted rhythm.)

In the auricle the normal auricular rhythm is

for the most part uninterrupted, although occasional retrograde stimulation of the auricle by the ectopic ventricular beats, and occasional ectopic auricular contractions, can be made out in the original electrocardiographic tracings. The normal cycle shows auricular systole to be followed by two distinct negative pressure waves forming the "W" contour mentioned above (see
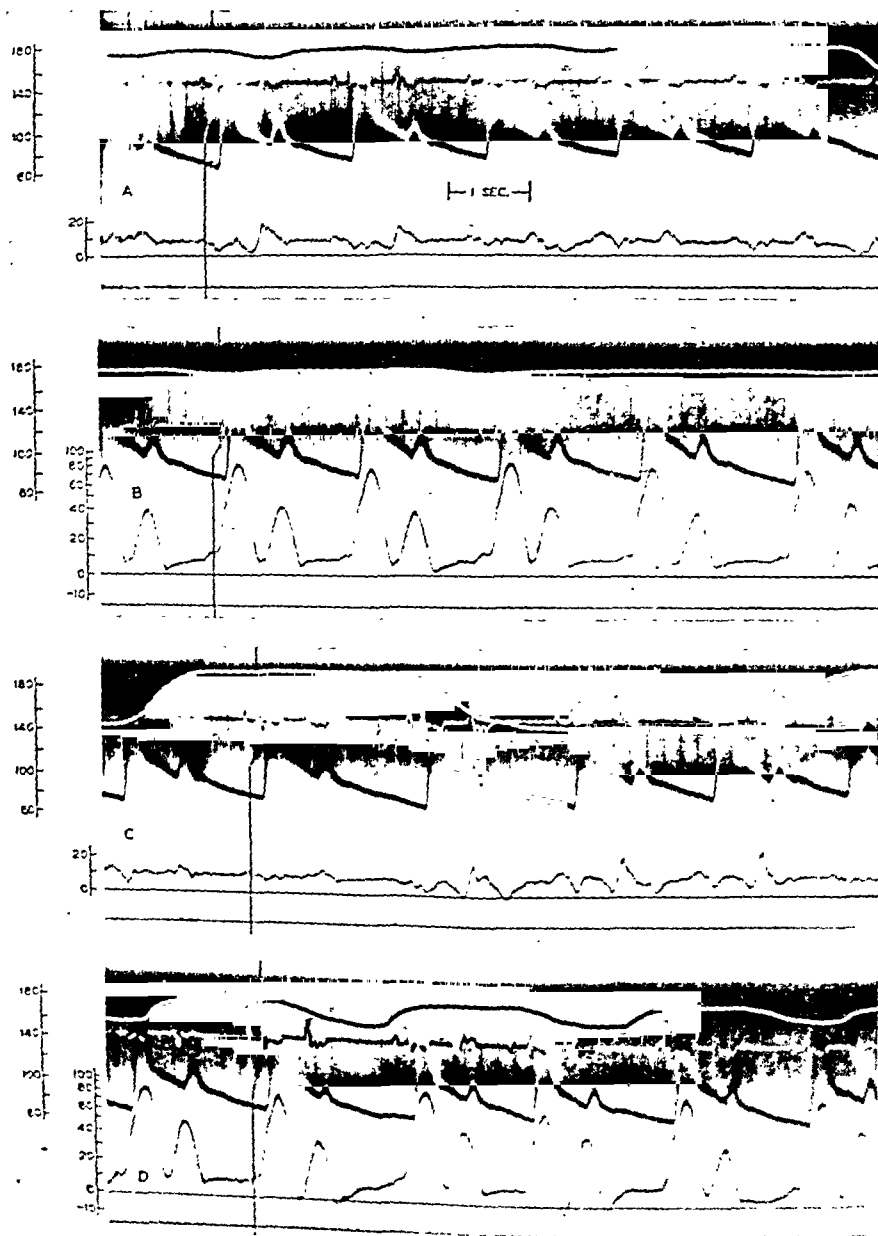
Fig. 7. Third Study of Patient No. 39, Again in Right Heart Failure.
A, auricle, and B, ventricle, during quiet respiration; C and D, during deep breathing. Electrocardiogram has been retouched in C and D; femoral pulses are obscured by overexposure. Conducted beats have average systolic pressure of 81 mm. Hg. and pulse pressure of 69 mm. Hg; ectopic beats have average systolic pressure of 41 mm. Hg, and pulse pressure of 31 mm. Hg. It is obvious from femoral and ventricular curves that the ectopic beats are "abortive" insofar as ventricular output is concerned. Detailed description in text.
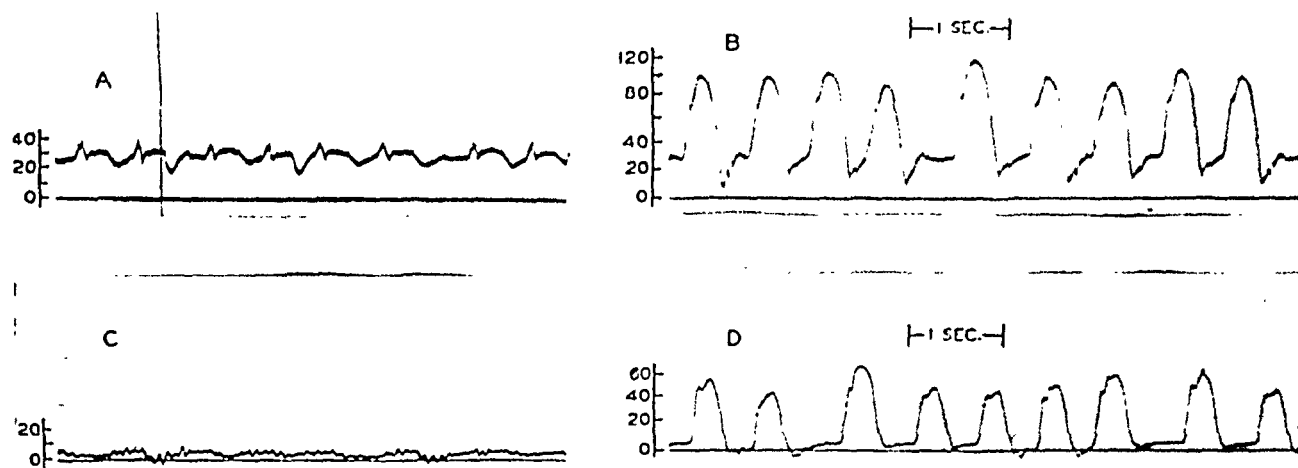
FIG. 8. PATIENT No. 51, RHEUMATIC HEART DISEASE.
A, auricle, and B, ventricle, from first study at time of right and left heart failure. C, auricle, and D, ventricle, from second study, when compensation had been restored. Vertical line in A is crack in film. See text for details.

Figure 7C). The first is due to descent of the base of the heart at the time of the more vigorous post-extrasystolic ventricular contraction; the second is the fall which is characteristically prominent in the presence of right ventricular failure, and is due to rapid auricular emptying early in diastole. The effect of the ectopic ventricular beat on the auricular pressure curve, coming at the time of rapid auricular emptying in the preceding cycle, is striking. The auricular pressure, rising again as the chamber refills from the venous system, is further increased by impact of the premature beat, which occurs at a time of such high velocity flow into the auricle that the effect may be likened to that of a water-hammer. Presumably this weaker ventricular beat occasions less descent of the cardiac base. In addition, the next normal auricular contraction takes place during the course of the impact from the ventricular extrasystole. These factors, auricular refill, extrasystolic impact, incomplete descent of the cardiac base, and auricular systole, all summate to produce the three tall spikes so clearly seen during deep inspiration in the right-hand portion of Figure 7C, during which phase auricular filling from the veins is probably at a maximum.

2. Rheumatic heart disease, with mitral insufficiency and stenosis, aortic insufficiency, tricuspid insufficiency and auricular fibrillation. In Figure 8 are presented the 2 studies of patient No. 51. The auricular and ventricular curves from the first study (Figure 8A and B), recorded when the pa-

tient was in left and right heart failure, are typical. An additional feature of the auricular record is its evidence of tricuspid insufficiency, which is discussed in a subsequent section. The mean auricular and end-diastolic (P_{d_2}) ventricular pressures are both 29 mm. Hg. The ventricular systolic pressures are at the unusual average height of 103 mm. Hg, or almost 4 times normal, with considerable individual variation due to the irregular ventricular rate. The early diastolic dip is marked. In the second study (Figures 8C and D) compensation had been restored following treatment. The mean right auricular pressure is normal, and although the tracing is technically not satisfactory for detailed analysis, there is no longer any evidence of tricuspid regurgitation. The ventricular diastolic pressure is close to zero; the early diastolic dip is barely descernible in some cycles (as in normal curves) and absent from most. The ventricular systolic pressure, though much lower than before, is still at the abnormally high figure of 57 mm. Hg. Two factors may account for such residual systolic hypertension: the persistent congestion of the left auricle and pulmonary vessels behind the more or less fixed barrier of the mitral lesion, and the possible coexistence of secondary pulmonary vascular disease of the type emphasized by Parker and Weiss (15). The pressures now are similar to those found in patient No. 50, already discussed, who had never been in congestive heart failure.

3. Arteriosclerotic heart disease. In Figure 9A

are a pair of tracings from patient No. 58, recorded through the double-lumen catheter. The left hand portion of the film consists of a peripheral venous tracing (upper) taken simultaneously with an auricular tracing (lower). The auricular channel of the catheter was then quickly attached to the manometer previously connected to the peripheral

vein, and the ventricular channel was transferred to the manometer previously connected with the auricle. The right hand portion of the film was then inscribed. Thus it was possible in rapid sequence to use one instrument for comparative recording of vein and auricle, and another for a similar comparison of auricle and ventricle. It is ob-
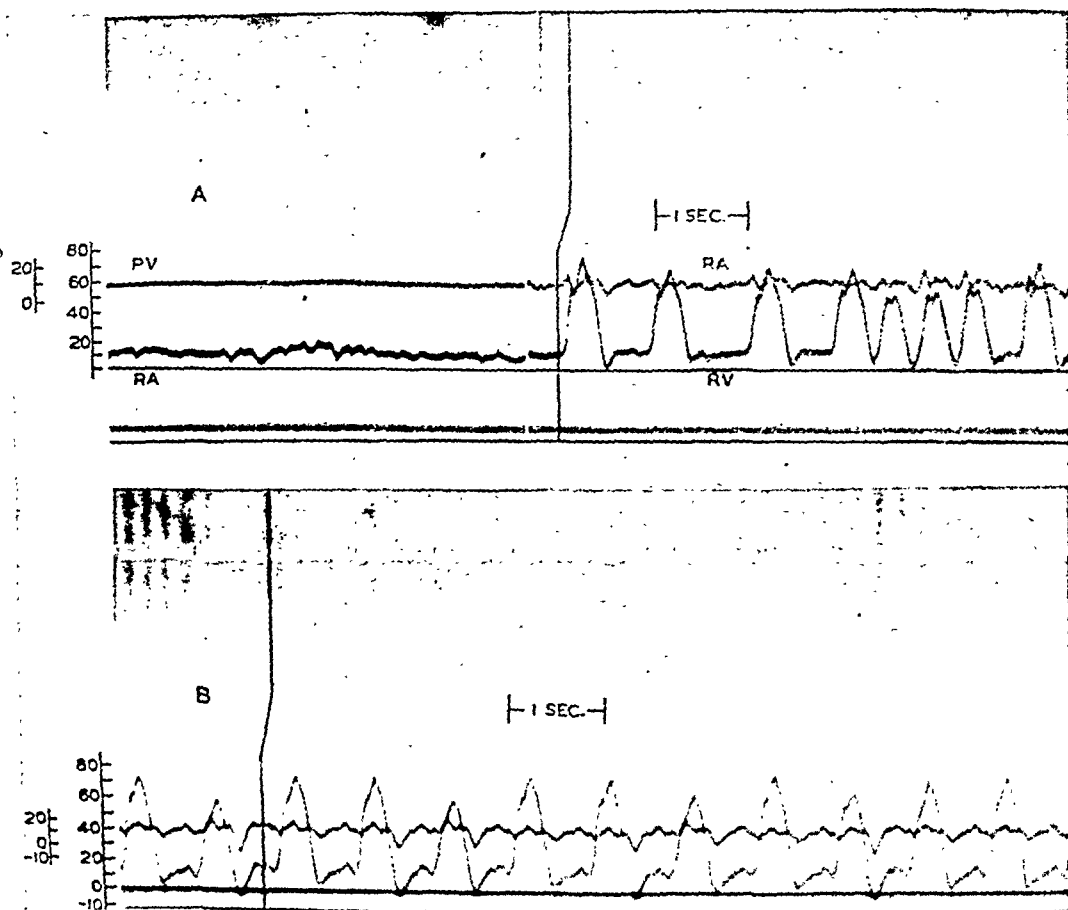
Fig. 9. A. Patient No. 58, Arteriosclerotic Heart Disease, in Failure

Left, simultaneous peripheral venous (PV) and right auricular (RA) pressures; right, right auricular (RA) and right ventricular (RV) pressures, recorded through double lumen catheter (5). The two auricular tracings are not directly comparable because of different sensitivities of manometers. Small scale applies to PV, left, and RA, right; other scale applies to RA, left, and RV, right. Coarse "f" waves of electrocardiogram appear to correspond to waves in the auricular pressure record. Due to auricular fibrillation, ventricular beats vary in amplitude. Where diastole is short, rising pressure phase of ventricular diastolic curve, i.e., the second portion of the dip, is abbreviated and merges with onset of next contraction. In cycles where dip is prominent, note that it corresponds to rapid emptying phase of auricle. On auricular curve, where ventricular rate is rapid, the same summation of ventricular impact plus refill from veins is seen that was pointed out in case of terminal rhythm shown in Figure 7; auricular systole, however, is not present.

B. Syphilitic Heart Disease

Simultaneous auricular and ventricular curves from patient No. 59, in whom right heart failure is secondary to left ventricular failure. Curves are similar to those of right ventricular failure due to increased pulmonary resistance of other etiologies.

vious on inspection how close in value are the mean auricular and peripheral venous pressures, which by planimetry were each 10.5 mm. Hg. The correspondence between the auricular and the ventricular diastolic pressures is also apparent. As has been pointed out, the diminution in gradient from peripheral vein to auricle has been a feature of right heart failure, even in the absence of a markedly elevated peripheral venous pressure (13, 14). Forward flow in the vein under consideration may still be accomplished, in spite of no mean venous-auricular gradient, as long as the auricular pressure at some time in the cardiac cycle is below that in the vein, as is especially true early in ventricular diastole. The early ventricular diastolic dip and its counterpart in the emptying phase of the auricular record are less prominent than in the preceding case (Figures 8A and B) in which the central venous pressures were much higher. In other respects, however, the auricular and ventricular tracings show the salient features already discussed as exemplifying right heart failure.

4. Syphilitic heart disease, with aortic aneurysm and free aortic regurgitation. Tracings from patient No. 59 are shown in Figure 9B. The initial clinical manifestations were the classical ones of acute left ventricular failure; this was the patient's second episode of decompensation. There was no evidence of pulmonary disease or arteriosclerosis. At the time of study he was in frank right and left heart failure; the cardiac output was only 88 per cent of average normal at a time when the oxygen consumption was 45 per cent greater than the average basal value. The right heart and ventricular pressure pulses, recorded simultaneously through the double-channeled catheter, are characteristic of right heart failure.

5. Heart disease of unknown etiology. Two patients with right ventricular failure of undiagnosed cause were studied. The first, No. 60, was a 48-year-old female, whose history extended over several years and included several hospital admissions. During the first admission she was found to have arterial hypertension associated with left ventricular failure. On a subsequent entry she was suffering from a respiratory infection, which was complicated while in the hospital by a sterile pericardial effusion. At the time of her third admission she was in right- and left-sided failure.

On conventional treatment compensation returned and the first hemodynamic study was then carried out. In addition to the essentially normal right heart pressures shown in Figure 10A, minimal systemic hypertension was present as evidenced by a mean arterial pressure of 112 mm. Hg. The cardiac index was 2.33 L. per minute per sq. m., which is 75 per cent of average normal. On the tracing there is no definite early diastolic dip in the right ventricular curve, diastolic pressure remaining close to zero and varying significantly only with respiration. The right auricular emptying phase is associated with a pressure fall of only
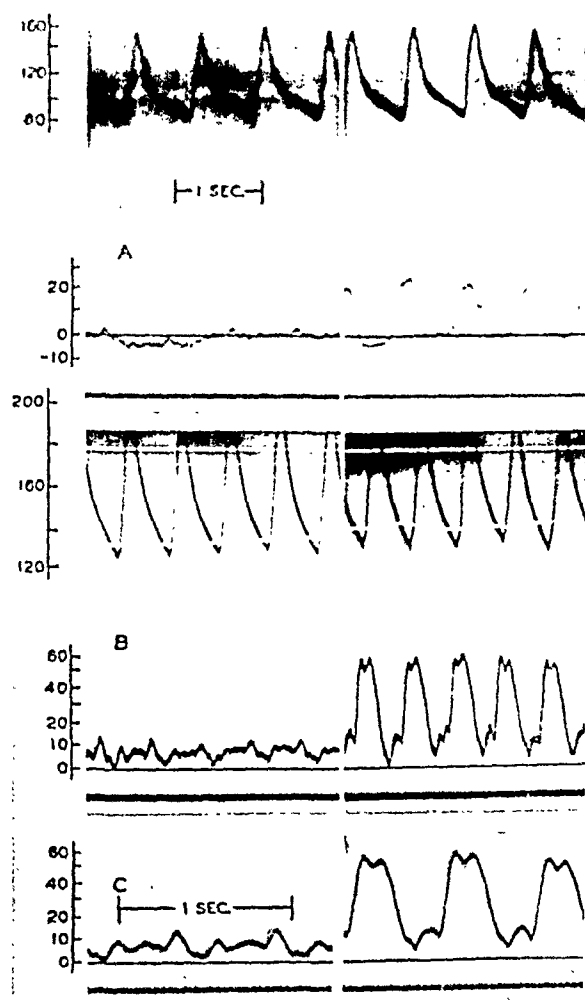
Fig. 10. A. First Study of Patient No. 60, When Previous Heart Failure Was no Longer Present

Essentially normal record.

B. Second Study of Same Patient, at Time of Right and Left Heart Failure

Heart rate, 110. Significance of prominent auricular systole in shaping diastolic portion of ventricular curve is discussed in text.

C. High Speed Tracing Showing Details of B

1 or 2 mm. Hg. The second study of this patient was made on her next admission, at which time she was in right and left heart failure, with a cardiac output 64 per cent of average normal. The expected elevation of right ventricular systolic and diastolic pressures and of right auricular pressures as well as the deep early diastolic dip in the ventricular curve, were present (Figure 10B). Individual details of the cycle are shown more clearly on high camera-speed tracings of Figure 10C. Inspection of the line of decline of ventricular pressure following closure of the pulmonic valve, shows a clear-cut inflection near its base, as a new gradient of pressure marks the beginning (descending phase) of the dip. The ascending limb, and the remainder of diastole apparently corresponds to auricular systoles of unusually great amplitude. The abbreviated ventricular diastole cuts short the period of rapid auricular emptying and its coincident fall in pressure. An unusually vigorous contraction might therefore be expected from the auricle, since its incomplete emptying leaves it under greater stretch at the time its own systole begins. Such an explanation would ascribe to the auricular contraction, in this case, a greater than usual importance for ventricular filling.

The second patient, No. 61, a 37-year-old sailor with a long history of alcoholism, had been admitted several times in complete heart failure. The 2 diagnoses under consideration were active myocarditis of unknown etiology and beri-beri heart disease. During the present admission, in response to conventional therapy, all clinical evidence of heart failure had disappeared by the time the hemodynamic studies were made, but
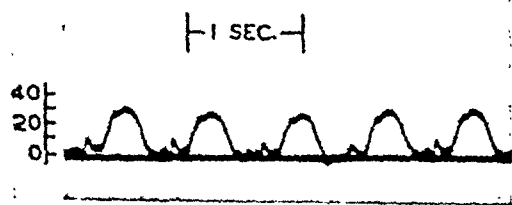
FIG. 11. PATIENT No. 61, HEART DISEASE OF UNKNOWN ETIOLOGY, NOT IN FAILURE

Pressure values are normal, but diminished gradient of systolic pressure rise is consistent with hypodynamic heart action.

roentgenograms showed persistent and marked cardiac enlargement. The cardiac output was only 74 per cent of average normal. Right heart pressures were normal. Since the mean pulmonary arterial pressure was probably normal, insofar as can be judged from the ventricular curve, and since the cardiac output was less than normal, the pulmonary resistance was presumably increased. It will be seen in Figure 11 that the right ventricular systolic pressure rises more slowly than in normal curves. This decrease in ventricular systolic slope, in the presence of elevated pulmonary resistance, presumably indicates a slower than normal development of mechanical energy by the ventricle. Such hypodynamism has been shown by Wiggers (20) to occur in hearts damaged experimentally by toxins, inadequate coronary blood supply, agents such as chloroform, etc.

Comment

It would appear from the varied group of cases presented that when increased pulmonary resistance is the cause of clinical right heart failure, pressure pulses from the right heart are generally similar, except when tricuspid insufficiency modifies them, regardless of the etiology or localization of the responsible factor. As might be expected, the pressure pulse records, after compensation is restored, vary from case to case, depending on the degree of residual obstruction to the pulmonary blood flow, and on the stroke volume. Thus in patient No. 51, marked systolic hypertension remained, and the right ventricular curve was similar to that found in patient No. 50 with mitral stenosis who had never been in right heart failure. Both patients with heart failure of uncertain etiology, studied after disappearance of all clinical evidence of heart failure, had normal right heart pressures. However, the cardiac output in both was sufficiently low to suggest that pulmonary resistance was still somewhat above normal. It should be emphasized that in order to estimate resistance, both pressure and flow must be known. Therefore, where a knowledge of cardiac output is lacking, an isolated normal right ventricular pressure record must be interpreted with caution in evaluating the pulmonary resistance, since normal pressures may be found in the presence of obvious and severe heart disease, as evidenced by these 2 patients.

Properly speaking, in order to measure pulmonary vascular resistance, the mean pressure in the pulmonary artery should be known. Experience with simultaneous recording of pulmonary and right ventricular pressure is very limited. The most satisfactory tracing so far obtained, from patient No. 57, a case of rheumatic valvulitis in cardiac failure, is shown in Figure 12.

III. Tricuspid insufficiency

An unusual opportunity has been afforded to observe the auricular pressure pulses in 8 cases of tricuspid regurgitation; in 4, ventricular curves were also recorded. Seven of these patients, Nos. 51 through 57, had rheumatic heart disease with multivalvular lesions, and 1, No. 62, had congenital heart disease with a probably large inter-auricular septal defect. All had auricular fibrillation, and all were in heart failure. The tricuspid regurgitation had been clinically suspected in all of the rheumatic cases; the congenital heart case

has been added to the group because the tracings were considered diagnostic of the lesion. The heart failure aspects of the records in one patient (No. 51, Figures 8A and B) have already been cited.

Figure 13 from patient No. 52 is an unusually good example of tricuspid insufficiency in the presence of left and right heart failure. In the upper half of the figure, the right auricular and ventricular pressures were recorded simultaneously without parallax by means of the double-lumen catheter. By this technique, the events of the auricular curve can be easily compared to those of the ventricle. Auricular systole is absent because of fibrillation. At the onset of ventricular systole, the auricular curve displays a brief spike-like rise of pressure, which is probably due to the impact from the contracting ventricle. Concomitant with the downstroke of this spike, there is a slur in the ventricular upstroke, the nature of which is uncertain. It has been ob-
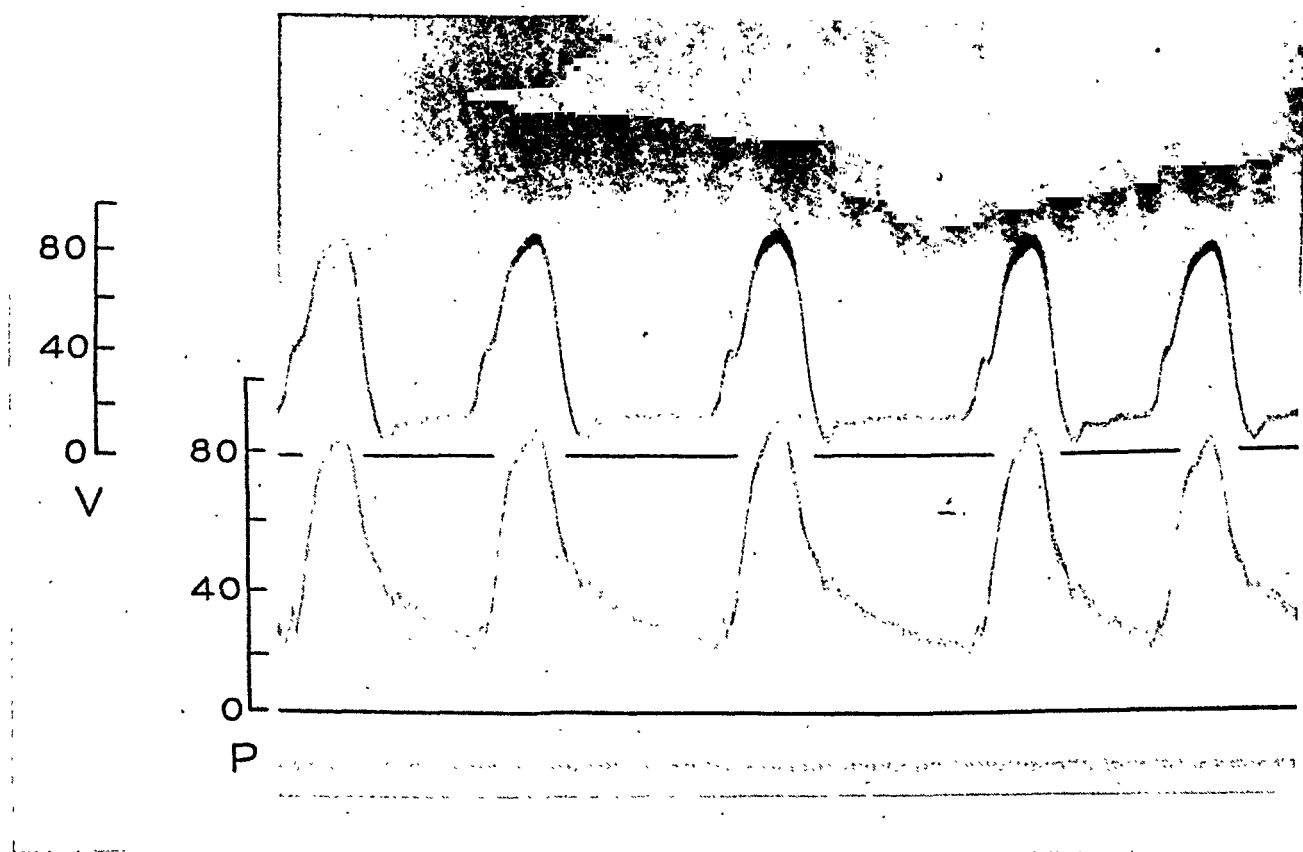
FIG. 12. RIGHT VENTRICULAR (UPPER) AND PULMONARY ARTERIAL (LOWER) PRESSURES IN A CASE OF LEFT AND RIGHT HEART FAILURE (PATIENT No. 57)

Note (a) elevation of systolic as well as ventricular diastolic pressures, (b) identity of contour during most of systole, and (c) identity of maximum systolic values in both tracings. Mean pulmonary arterial pressure is 48 mm. Hg, estimated to be about 3 times normal.

served in all 4 cases of tricuspid insufficiency in which ventricular catheterization was successful (Figures 8B and 12). In the auricle the initial systolic spike is followed by a phase in which the curve is plateau-like or slightly convex upward, representing regurgitation from the ventricle.

Two distinctive features of this plateau are (*a*) that its pressure level is *higher* than that prevailing during the presystolic interval (instead of lower, as in normal subjects and in patients with a competent tricuspid valve) and (*b*) that it is sustained exactly until the end of isometric relaxa-
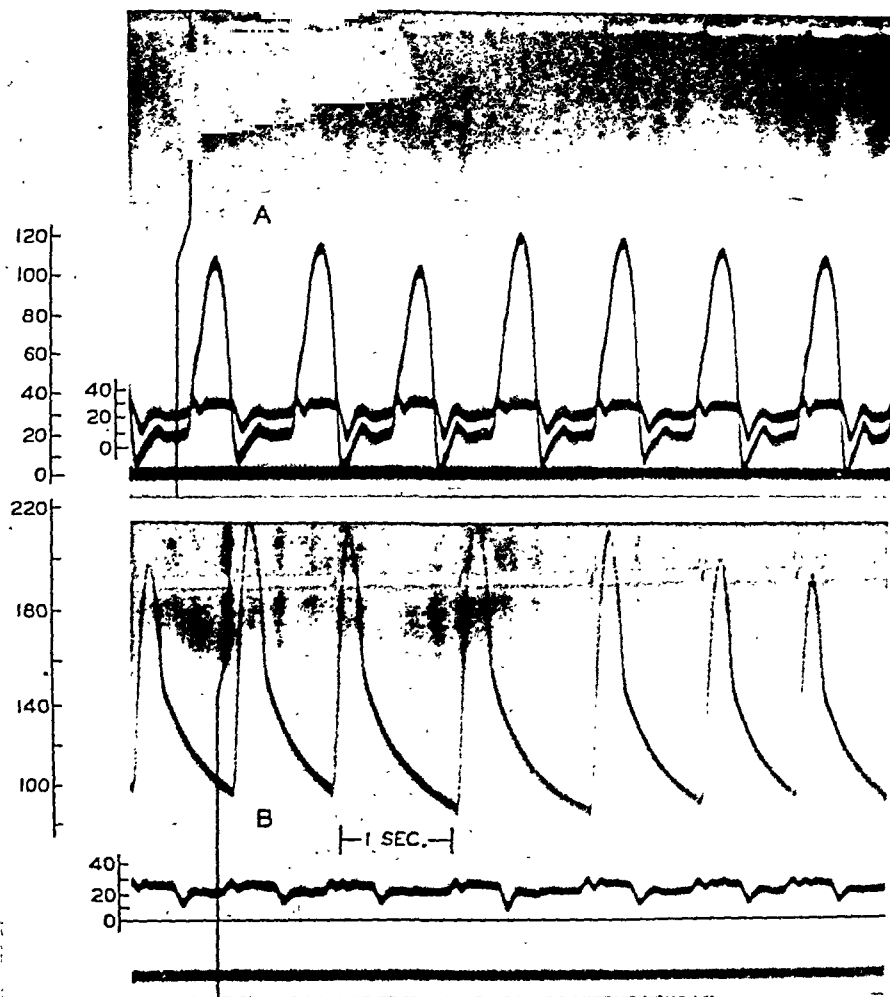
FIG. 13. PATIENT No. 52, RHEUMATIC HEART DISEASE, WITH RIGHT AND LEFT HEART FAILURE AND TRICUSPID INSUFFICIENCY

A. Simultaneous auricular and ventricular pressures recorded through double lumen catheter.

B. Femoral arterial and right auricular pressures. Typical features of tricuspid insufficiency are seen in auricular curves; relationship between auricular emptying phase and early ventricular diastolic dip is especially well shown. Maximum ventricular systolic pressure averages 116.5 mm. Hg, about 4 times normal. The most marked degrees of ventricular hypertension seen were in this patient and in No. 51, both of whom had severe mitral lesions. See text for details.
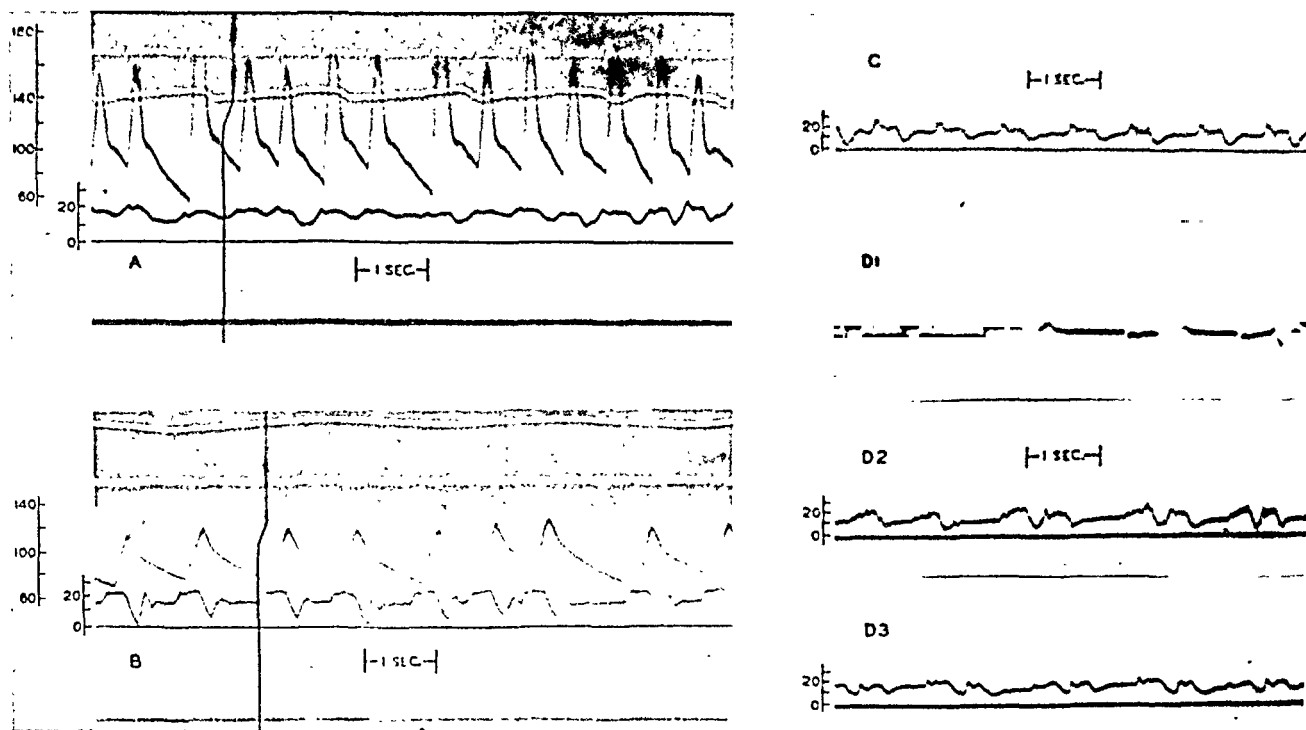
FIG. 14. RIGHT AURICULAR RECORDS FROM PATIENTS No. 53, 62, 54, AND 55, RESPECTIVELY, SHOWING TRICUSPID INSUFFICIENCY

Note similarity of all to auricular pattern of Figure 13. Contrast with normal curves in Figure 1. Further description in text.

tion, at which instant the ventricular pressure falls below the auricular level and the tricuspid valves open. As the pressure in the ventricle continues to fall as a result of the relaxation of its muscle fibers, the auricular pressure also drops, but at a slower rate, and reaches its lowest value 0.05 to 0.06 second *after* the ventricular pressure has fallen to its minimum value. Not only does the ventricular pressure fall faster, but it also decreases to a level averaging about 6 mm. Hg lower than that in the auricle. These facts suggest that, in tricuspid insufficiency, at least, it is the relaxation of the ventricle itself which initially creates and momentarily sustains the gradient of pressure that results in the rapid filling of this chamber early in diastole (16). Whether this is to be considered a true aspiration in the sense that the relaxing ventricular wall exerts an outward or enlarging force aspirating blood from the auricle, can probably not be determined from the 2 pressure curves alone. However, Katz and Brams have presented evidence that such is the case in the isolated turtle heart (17) and in the dog (18, 19). The inrush of blood from the auricle under the venous pressure head quickly halts the ventricular pressure fall and then reverses it, whereupon the pres-

sures in the two chambers approximately equalize and rise rapidly together to complete the early diastolic dip. During the rest of diastole the pressures are stabilized at the venous level.

The height of the ventricular pressure reached during systole in this case is remarkable, being about 4 times greater than normal.

In Figure 13B, the auricular tracing was recorded after the tip of the catheter had been withdrawn from the right ventricle. The characteristic features of tricuspid insufficiency are even more clearly shown than in Figure 13A. Since both auricular tracings of Figure 13 are practically identical, it is apparent that in this case the presence of the catheter in the tricuspid valve was not the cause of regurgitation. The femoral arterial pressure curve shown in Figure 13B is consistent with the very high peripheral resistance present in this patient (arterial hypertension in spite of subnormal cardiac output).

At post-mortem 3 months later, very marked hypertrophy and dilatation of the right ventricle were found, the walls measuring as much as 1.2 cm. in thickness in some areas. The mitral valve orifice was extremely narrow. The left ventricle was neither hypertrophied nor dilated. Although

some thickening of the tricuspid valves at the line of closure was noted, anatomical diagnosis of organic tricuspid insufficiency was not included in the autopsy report.

In Figures 14A, B and C (patients No. 53, 62 and 54), the characteristic features of tricuspid insufficiency are clearly seen, and require no further comment.

In case No. 55 (Figure 14D), where there might well be some doubt as to interpretation of the tracing, an electrocardiogram (D1) taken the same morning revealed long periods of complete heart block during which regularly recurrent ectopic ventricular beats took place, giving rise to bigeminy. The time relationship of the nodal and ectopic beats on the electrocardiogram was found to correspond to the rhythm of the coupled beats on the pressure record. It was thus possible to identify each of these phases with a separate cycle, and to ascertain that each cycle had all the characteristics described for tricuspid insufficiency. Tracing D3 of this figure was taken after the catheter had been withdrawn to the periphery. Except for some damping, it shows a remarkable retrograde transmission of the auricular pressure variations (D2). It should be noted, however, that D3 represents *end* pressures, since no flow occurred past the portion of the catheter lying in the vein of the arm. The peripheral venous curve (not shown), recorded through a needle in the opposite vein, was much more damped.

Peripheral venous and auricular mean pressures were markedly elevated in the whole group. The existence of a mean pressure gradient which was positive from right auricle to peripheral vein is of considerable interest in cases No. 51, 54, and 55. The same order of reversed gradient was observed when the respective pressures were measured by saline manometers. It must therefore be inferred that intermittent backward flow in the direction of the distended arm veins takes place, and that a pulsating forward flow of greater magnitude into the right auricle and ventricle occurs early in diastole when the pressure in these chambers falls well below the venous pressure.

Comment

As is true in right ventricular failure due to elevated pulmonary vascular resistance, the diagnostic pattern of the tracings in tricuspid insufficiency is attributable to a functional abnormality, independent of etiology or lesion. Though the fact of regurgitation seems established, the tracings do not serve to distinguish between insufficiency due to passive dilatation of the valve ring, and that due to organic valve disease. In the rheumatic group either cause or both might have been responsible. One additional point of some diagnostic interest was observed in this study. The attempt to insert the catheter into the ventricle was successful in only half of the cases. In patients No. 53 to 55, and 62, each time the catheter was pointed in the proper direction to enter the valve, as seen under the fluoroscope, ventricular contraction was observed to cause rejection of the catheter tip which flipped away during systole and returned during diastole to a position pointing toward the valve. This has not been the case in other lesions, and is regarded as evidence of regurgitant flow through the A-V valve at the time of right ventricular systole.

In general, the auricular tracings from cases of tricuspid insufficiency resemble published jugular venous pulse records from patients having this functional defect (20).

Because considerable attention has been given to the description of the early diastolic "dip," it may be well to discuss briefly some points related to its physiological significance: (a) Its occurrence in the auricular and ventricular pressure curves is believed to be of ventricular origin, because the downstroke has a steeper slope and reaches a lower level of pressure in the ventricle than in the auricle. There can be little doubt as to the correctness of this view in the cases of tricuspid insufficiency (Figures 8 and 13), and probably also in the cases of right heart failure without tricuspid insufficiency (Figure 9B). Extension of the same reasoning to the cases with a normal central venous pressure is questionable, since in these cases the ventricular dip may be due, at least in part, to catheter motion. It may be mentioned, however, that the corresponding pressure fall in the auricle (Figure 1A) is accepted in animal tracings as a true pressure record and is attributed to opening of the A-V valves and flow of blood into the ventricle (7). The greater amplitude of the dip during inspiration, when the intrathoracic pressure is lower and therefore the inflow into the right heart larger, is consistent with the

above interpretation. (b) When the venous system and right heart are markedly distended, as in congestive failure with tricuspid insufficiency, small changes in volume will be accompanied by large changes of pressure, the situation being probably analagous to that described for veins by Ryder and Ferris (21). Therefore, when the ventricle relaxes and the auricle empties into the ventricle, the pressure in both chambers falls much farther below the general venous level than in normal cases, although the associated volume displacement is less than normal. Hence, a dip of great amplitude occurs (Figures 8, 13, and 14). In cases of congestive heart failure without tricuspid insufficiency, the same mechanism explains the greater amplitude of the systolic dip in the right auricular tracings, associated with descent of the base.

IV. Constrictive pericarditis

Auricular and ventricular pressure pulse records were taken as part of a more comprehensive study of a young male with the classical clinical picture of constrictive pericarditis. The tracing resembled

those described in the section on right ventricular failure without tricuspid insufficiency; however, certain additional features of diagnostic significance were present, which in conjunction with the electrocardiogram and other circulatory measurements form the basis of a forthcoming report. The most obvious of these, as seen in Figure 15, are the existence of a virtually normal ventricular systolic pressure, a low ventricular pulse pressure, a marked elevation of the mean auricular and ventricular diastolic pressures, prominence of the early diastolic dip in the auricle and ventricle, and a marked fall in auricular pressure during ventricular ejection, which, with the diastolic dip, give to the tracing a distinct "W" form.

V. Arterial hypertension

Pulse tracings from the right ventricle were essentially normal as to contour and pressure values in the small group shown in Table I, none of whom presented any evidence of either left or right heart failure The mean femoral arterial pressures of 114, 132, 150, 114, and 128 are all above the normal upper limit taken to be about 110 mm. Hg. The right ventricular systolic pressures of 21 to 30.5 mm. Hg are within the range of normal. The diastolic pressures and, by implication, the auricular pressures, are also quite normal, as would be expected. The right ventricular pulse pressure of 28.5 mm. Hg in patient No. 67 is only 2 mm. above the highest figure found in the normal series. The results of the present limited study suggest, therefore, that increased systemic arteriolar resistance has no counterpart in the pulmonary circulation; however, definitive conclusions in human essential hypertension should await further investigation.

VI. Shock

Although the methods used in the study of the patients in the present paper have been routinely employed in many cases of shock (22, 23), almost all of the intracardiac pressure curves were technically unsatisfactory. Mean pressure values obtained from auricular records seem to be reliable, when compared with simultaneous readings on saline manometers, but the details of the auricular cycle were for the most part badly obscured by oscillations which were probably artefacts.
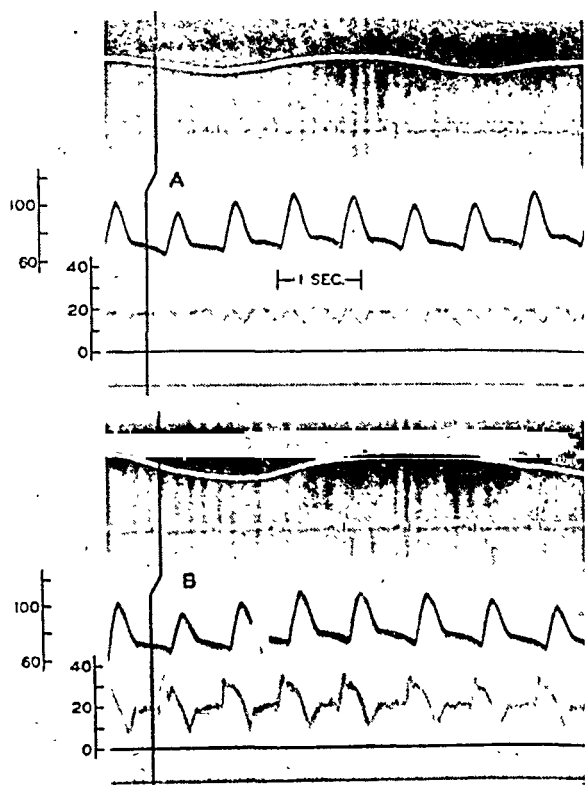
FIG. 15. PATIENT No. 64, CONSTRICTIVE PERICARDITIS
See text for details.

Ventricular pressure curves were even less adequate except in 3 instances: (*a*) in patient No. 70, a case of chronic anemia due to a bleeding peptic ulcer, who developed signs of mild shock at the time of an acute hematemesis, and in whom the ventricular systolic, diastolic, and pulse pressures were all very low; and (*b*) in 2 patients in whom acute hypotensive states were deliberately but briefly induced by controlled phlebotomy. Figure 16 summarizes one of these studies in patient No. 13, a small male, 53 years of age. A rapid 600 ml. phlebotomy was followed, after a brief interval, by infusion of 650 ml. of 5 per cent gelatin solu-

tion. The dramatic fall in arterial pressure, associated with marked pallor and a decrease of cardiac output to 40 per cent of the control value, was concomitant with a pronounced reduction in right ventricular pressures, as might be expected. On recovery from the acute collapse the pressures in the ventricle and the systemic arteries returned simultaneously toward normal.

VII. Pharmacodynamic studies

During the study of shock, observations have been made on the circulatory effects of two pressor amines, both on normal control subjects and on pa-
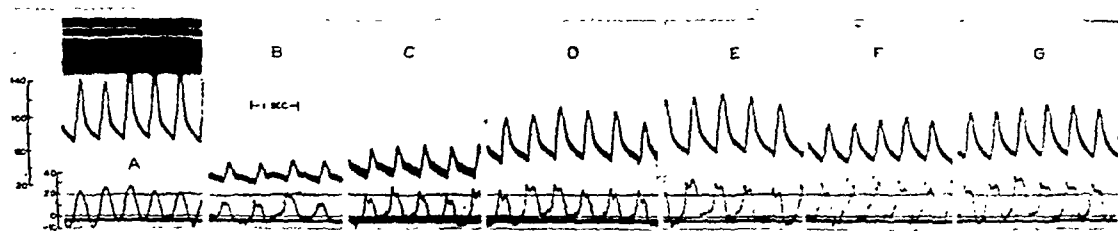
FIG. 16. INDUCED HEMORRHAGE IN PATIENT No. 13

A, control femoral arterial and right ventricular (damped) pressures.

B, same, 5 minutes after 600 ml. of blood were removed in 13 minutes; marked pallor, reduced heart rate; cardiac output 40 per cent of control.

C, 20 minutes after phlebotomy; 200 ml. of 5 per cent gelatin in.

D, 38 minutes after phlebotomy; 390 ml. of gelatin in.

E, 57 minutes after phlebotomy, 600 ml. gelatin in.

F, 86 minutes after phlebotomy; 25 minutes after end of 650 ml. gelatin infusion; cardiac output now 89 per cent of control.

G, 234 minutes after phlebotomy, 173 minutes after infusion; cardiac output now 95 per cent of control.
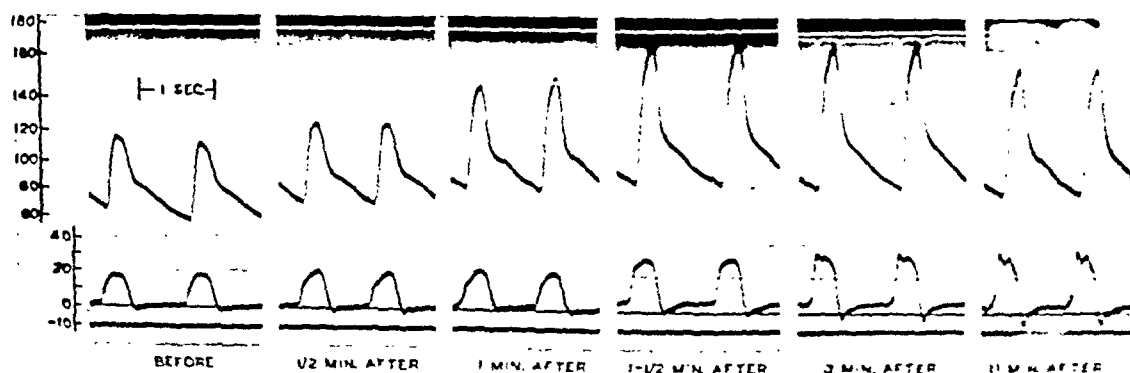
FIG. 17. EFFECT OF METHEDRINE IN PATIENT No. 3

Each section was taken from same phase of respiratory cycle. Dose was 3 mgm. intravenously. Note rapid and concurrent rise in systemic and right ventricular pressures. At 1½ minutes, the ventricular diastolic pressure and by inference, auricular and central venous pressures, also have increased appreciably. Thus, pressure is elevated throughout the circulatory system. A dip appears at 1½ minutes, concomitant with increased rate of ventricular relaxation, as judged from steepness of descending curve of the isometric relaxation period. Dip is probably exaggerated in last 2 sections by a shift in respiratory respiration. Three minutes after last pressure recording, cardiac output was 117 per cent of control.

tients suffering from clinical shock. Since changes may occur rapidly after administration and since important changes may be transitory, it is essential to make early and frequent determinations after the control period in order to follow the true sequence of events. Serial tracings made in patient No. 3 following administration of "methedrine" are shown in Figure 17 to illustrate a type of systemic and right heart pressure response that may occur. Such recordings, when taken in conjunction with cardiac output and other measurements, provide a more complete picture of the response to a drug than has been hitherto available in clinical investigation.

VIII. Effects of respiration

An impressive feature of many of the tracings, particularly in the group of patients with disease of the thorax or lungs, has been the rôle played by the variation in intrathoracic pressure in de-/ termining intracardiac pressure levels and blood flow. Not only is this factor of importance in hemodynamic considerations, but it is clear that the varying intrathoracic pressure is physiologically a more appropriate base-line from which to measure intracardiac pressures than the atmospheric pressure which it is usually necessary to use (Figure 1C). Figure 5 illustrates a striking influence of deep respiration on the pressures. An example of the effects of cough on the intrapleural, auricular and ventricular pressures is illustrated in Figure 18. A more extended analysis of the respiratory influences on intracardiac pressures will be reported later (12).

SUMMARY

1. A method is described for recording, singly or simultaneously, the pressures in the right auricle and right ventricle.

2. The method was used in 70 patients without undue discomfort or complication.

3. The form of the tracings and the range of pressure variations are described in 17 normal subjects and 53 patients with the following clinical conditions: chronic pulmonary emphysema, fibrosis, or both, with and without signs of cardiac failure; post-pneumonectomy; fibrothorax; kyphoscoliosis; pneumothorax; rheumatic heart disease, with and without signs of cardiac failure; arteriosclerotic heart disease and syphilitic heart disease with cardiac failure; heart disease of uncertain etiology; congenital heart disease; constrictive pericarditis; arterial hypertension without cardiac failure; and peripheral circulatory failure.
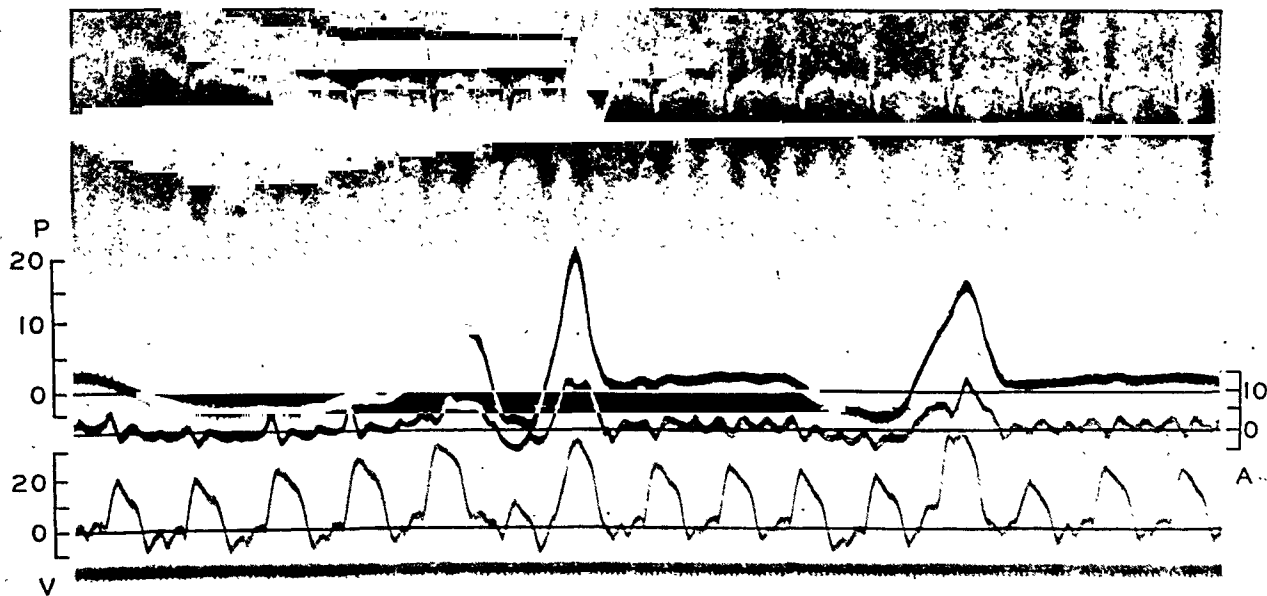
FIG. 18. ILLUSTRATING EFFECT OF SHARP COUGHS ON INTRAPLEURAL (UPPER), AURICULAR (MIDDLE), AND VENTRICULAR (LOWER) PRESSURES IN PATIENT No. 46

Corresponding calibration scales are labelled P, A, and V, respectively. Note that changes in right heart pressures are somewhat less than corresponding changes in intrapleural pressure.

4. The mean right auricular pressure in normal subjects varies from − 2 to + 2 mm. Hg, relative to atmospheric pressure. The normal right ventricular systolic pressure ranges between 18 and 30 mm. Hg, and averages 25 mm. Hg. The difference between the systolic pressure and the pressure at the end of diastole, termed the ventricular pulse pressure, ranges from 17 to 26.5 mm. Hg, and averages 22.5 mm. Hg.

5. The existence and approximate magnitude of pulmonary arterial hypertension can be determined in cases in which the right ventricular systolic and pulse pressures are increased.

6. The right ventricular systolic and pulse pressures are elevated in most of the patients with chronic pulmonary disease, but are normal in some cases of advanced pulmonary emphysema. These pressures are markedly elevated in all the cases of primary left heart failure, regardless of etiology.

7. Characteristic patterns of the pressure records in right ventricular failure due to various causes and in tricuspid insufficiency are described and partially interpreted.

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STUDIES ON BACTERIA DEVELOPING RESISTANCE TO PENI-CILLIN FRACTIONS X AND G *IN VITRO* AND IN PATIENTS UNDER TREATMENT FOR BACTERIAL ENDOCARDITIS

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Although penicillin G is the fraction of penicillin which is best known and most used, 2 other known fractions of this antibiotic have been isolated in crystalline form, penicillin F and penicillin X. Since the latter fraction is now being manufactured in appreciable quantities, it is important to determine whether it is superior in any way to penicillin G. We have attacked this problem by attempting to answer the following questions:

(*1*) What are the relative sensitivities of different bacteria to penicillins X and G?

(*2*) If the resistances of bacteria are raised *in vitro* by the use of penicillin X or penicillin G, what effect would this have upon the ultimate sensitivities of the organisms to the 2 fractions?

(*3*) If the resistance of an organism should be raised during the course of treatment of a patient with 1 of these fractions of penicillin, would there be any change in the relative sensitivity of the organism to the 2 fractions?

(*4*) Is there any difference in the absorption, distribution and excretion of penicillin X as compared with penicillin G?

(*5*) Is there any difference in the clinical results obtained with the use of the 2 fractions?

The last 2 questions have been discussed in another publication (1). In the present paper we shall give the results which we have obtained in seeking answers to the first 3 questions.

MATERIALS AND METHODS

Several preparations of penicillin X were used. One commercial preparation contained 75 per cent of sodium penicillin X; others, 90 and 95 per cent.[1] Several brands of commercial sodium penicillin G, made by various manufacturers, were used. Crystalline calcium penicillin G, obtained from the Food and Drug Administration, was also employed. In a few of the tests, a preparation of crystalline sodium penicillin G[2] was compared with the crystalline calcium penicillin G, and found to give identical results.

The tests for penicillin sensitivity combined in each tube 2 reagents: (*a*) 0.2 from a series of 2-fold dilutions of penicillin, and (*b*) 0.5 ml. of a standard dilution of each micro-organism in a medium containing 0.2 per cent of horse blood. A 24-hour culture of staphylococci (or other bacteria which grew readily) in tryptose phosphate blood broth was diluted in the same medium to the 10^{-5} dilution; streptococci, to 10^{-4}; pneumococci and meningococci, to 10^{-3}; influenza bacilli, to 10^{-2}. To the final dilution to be used, 0.2 per cent of the horse blood was added, and 0.5 ml. aliquots transferred to tubes already containing the required dilutions of penicillin. After 18 hours incubation the end-point tube showing no hemolysis or turbidity was recorded as containing the minimal effective concentration of penicillin. The end-points were checked by streaking the last negative tube on a blood-agar plate.

To increase the resistance of the bacterial strains to penicillins X and G, each was first tested for sensitivity and then transferred serially through: (*1*) a tube of an appropriate medium which contained the greatest amount of penicillin X which would allow growth; (*2*) a tube of same medium containing the greatest amount of penicillin G which would allow growth; and (*3*) a control tube of the medium containing no penicillin. The media employed were tryptose phosphate broth in the case of staphylococci, brain-heart infusion broth for streptococci and meningococci, and the same medium plus 1 per cent agar for pneumococci. At 24-hour intervals (or, in the case of meningococci, at 48-hour intervals), 0.2 ml. of the control culture was transferred to another tube of control medium. If the organisms in the tubes containing penicillin had grown well, 0.2 ml. of the culture was transferred from each tube to another tube which contained double the concentration of the same fraction of penicillin. If they had not grown well, the transfer was made to a tube containing the same concentration as on the previous day. This procedure was continued until the organisms persisted in growing poorly when transferred in the same concentration of penicillin for 3 successive days. When this plateau had been reached in each of the fractions of penicillin, the experiment was terminated as far as that particular strain was concerned, and the sensitivities of

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[1] Supplied by the Lederle Laboratories, Inc.

[2] Supplied by Merck and Company

the organisms which had been grown in the 2 fractions of penicillin, and of the control organisms, were tested.

The bacteria used in determining sensitivities and for the elevation of resistance were obtained from patients with various kinds of infections, with the exception of a few of the staphylococci and non-hemolytic streptococci. These were cultured from the blood of healthy persons with a transient bacteremia following the extraction of teeth.

RESULTS OF STUDIES ON COMPARATIVE RESISTANCE

Several investigations have been reported in which the comparative resistance of certain organisms to penicillins X and G were determined. Welch and his associates (2) stated that the X fraction was more effective than commercial penicillin against 1 strain each of *Klebsiella pneumoniae, Bacillus cereus* and pneumococcus Type I. Libby and Holmberg (3) tested 1 strain each of 10 different bacteria, and found only slight differences in their relative sensitivities to the 2 fractions. Ory and his co-workers (4) studied 209 strains, and found that the streptococci, pneumococci, gonococci and meningococci were generally from 2 to 8 times as sensitive to penicillin X as to penicillin G, on a unit-for-unit basis, while the staphylococci, 3 strains of Friedlander's bacillus, and 1 of *Hemophilus influenzae,* showed essentially the same resistance to the 2 fractions.

We have placed our results in the same form (Table I) as was used by Ory, in order to make comparison easy. One hundred and one strains were tested for resistance to penicillins X and G. Commercial penicillin preparations were used in the case of 56; crystalline penicillin preparations, in the case of 14; and in the case of 31 strains all 4 of these preparations were employed. Inasmuch as no significant difference could be observed in the results obtained with the crystalline and commercial preparations of either fraction, no distinction has been made between them in the table.

When a comparison of sensitivity to penicillin X and penicillin G is made on the basis of units, most of the organisms were more sensitive to penicillin X. Only 4 strains were more sensitive to the G fraction: 1 strain each of staphylococcus and *Hemophilus influenzae* and 2 strains of *Streptococcus viridans.* Seventeen strains (including the single strain of *C. diphtheriae* tested) were equally sensitive to the 2 penicillin fractions, while 80 strains were from 2 to 32 times more sensitive to penicillin X than to penicillin G.

The antibacterial effects of the 2 fractions may be better compared, however, on the basis of weight. Each mgm. of crystalline penicillin G has a potency of about 1,650 units; each mgm. of crystalline penicillin X, approximately 900 units (2).

TABLE I

Comparative sensitivity of various bacteria to penicillin X and penicillin G

Sensitivity, arranged according to units	Staphylococcus	Beta-hemolytic streptococcus	Streptococcus viridans	Pneumococcus	Gonococcus	Meningococcus	E. typhi	H. influenzae	C. diphtheriae	All bacteria	Sensitivity, arranged according to weight
More sensitive to X											
32-fold		1				1	1			3	16-fold
16-fold		2	2			2				6	8-fold
8-fold		1		2		5	4			12	4-fold
4-fold	6	7	5	6		8		2		34	2-fold
2-fold	4	8	6		2	2	1	2		25	Same sensitivity to X and G
Same sensitivity to X and G	4	6	2	1	2	1			1	17	More sensitive to G 2-fold
More sensitive to G 2-fold	1		2					1		4	4-fold
Total	15	25	17	9	4	19	6	5	1	101	

TABLE II

Range of sensitivity of 101 strains of bacteria to penicillin X and penicillin G

Organism	Amount of penicillin required to inhibit growth of least susceptible strain and most susceptible strain	
	Penicillin X	Penicillin G
	units per ml.	*units per ml.*
Staphylococcus	3.6 to 0.006	14.3 to 0.01
Beta-hemolytic streptococcus Group A	0.09 to 0.0007	0.4 to 0.0007
Streptococcus viridans	0.7 to 0.0007	1.4 to 0.0004
Pneumococcus Types I, III, IV, VII, and XIV	0.1 to 0.001	0.09 to 0.006
Gonococcus	0.006 to 0.0007	0.01 to 0.0007
Meningococcus Groups I, II and II alpha	0.04 to 0.006	0.4 to 0.01
E. typhi	1.4 to 0.6	9.0 to 2.2
H. influenzae Type B	1.1 to 0.1	9.0 to 0.6
C. diphtheriae (one strain tested)	0.02	0.09

Accordingly, a given weight of penicillin G is approximately twice as potent in terms of units as the same weight of penicillin X. In the extreme right-hand column of Table I we have arranged the legend so as to compare the relative sensitivities of the strains according to the weights of the 2 fractions used in the tests. Measured in this way, the 2 fractions showed less difference in their effectiveness upon the various bacteria. Twenty-five per cent of the strains showed equal sensitiveness to the 2 fractions, 54 per cent of the strains were from 2 to 16 times as sensitive to X as to G, and 21 per cent were from 2 to 4 times as sensitive to G as to X.

The range of the sensitivities of the various organisms to the 2 penicillin fractions is shown in Table II. All strains of beta hemolytic streptococci, gonococci and meningococci were very sensitive to penicillin X, and usually to penicillin G also. Staphylococcus and streptococcus strains varied from resistant to very sensitive. The few strains of *Hemophilus influenzae* and *Eberthella typhi* investigated were moderately resistant to penicillin X, and even more resistant to the G fraction. The 1 strain of *C. diphtheriae* encountered was moderately sensitive to both fractions.

RESULTS OF RAISING THE RESISTANCE OF BACTERIA *in vitro*

Ten strains of staphylococci, 4 of pneumococci, 2 of meningococci and 1 each of alpha-hemolytic and beta-hemolytic streptococci were made more resistant to both fractions of penicillin. Table III shows the original sensitivities of these

TABLE III

Development of resistance to penicillin X and G in vitro

Organism	Source		Number of transfers made	Original sensitivity		Sensitivity after transfers					
						In penicillin X		In penicillin G		In control medium	
	Material	Disease				Sensitivity tested by using penicillin:					
				X	G	X	G	X	G	X	G
				units per ml.		*units per ml.*		*units per ml.*		*units per ml.*	
Staphylococcus	Pus	Abscess of leg	43	0.2	0.4	28.6	28.6	3.6	3.6	1.43	1.43
Staphylococcus	Blood	Transient bacteremia following tooth extraction	47	0.01	0.02	0.2	0.5	0.2	0.9	0.02	0.04
Staphylococcus	Blood	Transient bacteremia following tooth extraction	42	0.04	0.09	7.2	3.6	0.2	0.03	0.04	0.04
Staphylococcus	Pus	Abscess of skin	14	0.02	0.02	1.4	1.4	1.4	1.4	0.09	0.09
Staphylococcus	Blood	Endocarditis	18	0.02	0.02	0.9	0.9	3.6	3.6	0.18	0.36
Staphylococcus	Urine	Pyelitis	28	0.9	1.8	14.3	28.6	7.2	14.3	1.79	1.79
Staphylococcus	Blood	Transient bacteremia following tooth extraction	23	0.01	0.02	0.2	0.5	0.2	0.9	0.02	0.04
Staphylococcus	Throat	Acute pharyngitis	44	0.2	0.2	1.8	0.9	7.2	14.3	0.71	1.43
Staphylococcus	Throat	Acute pharyngitis	18	0.04	0.04	0.3	0.3	1.8	3.6	0.09	0.09
Staphylococcus	Sputum	Bronchiectasis	19	0.01	0.01	1.8	1.8	28.6	28.6	0.04	0.04
Pneumococcus Type I	Blood	Pneumonia	50	0.003	0.01	1.4	0.7	0.09	0.4	0.01	0.04
Pneumococcus Type III	Spinal fluid	Meningitis	50	0.01	0.04	0.7	0.7	0.09	0.4	0.02	0.04
Pneumococcus Type XII	Spinal fluid	Meningitis	36	0.01	0.09	0.2	0.2	1.4	1.4	0.01	0.04
Pneumococcus Type XII	Blood	Pneumonia	26	0.01	0.01	0.06	0.06	0.1	0.1	0.01	0.02
Beta-hemolytic streptococcus, Group A, Type II	Throat	Scarlet fever	37	0.001	0.01	0.7	1.4	0.4	0.7	0.006	0.04
Alpha-hemolytic streptococcus	Urine	Pyelitis	30	0.02	0.04	0.2	0.2	0.4	0.4	0.04	0.09
Meningococcus Group I	Spinal fluid	Meningitis	13	0.02	0.09	Not done 1.8	1.8	3.6	3.6	0.02	0.09
Meningococcus Group II	Spinal fluid	Meningitis	28	0.04	0.2	2.9	2.9	0.2	1.4	0.02	0.04

TABLE IV

Comparative sensitivity to penicillin X and penicillin G of bacteria after resistance was raised in vitro

Sensitivity arranged according to units	Before resistance raised to penicillin					After resistance raised										Sensitivity arranged according to weight
						to penicillin X					to penicillin G					
	Staphylococcus	Pneumococcus	Streptococcus	Meningococcus	Total	Staphylococcus	Pneumococcus	Streptococcus	Meningococcus*	Total	Staphylococcus	Pneumococcus	Streptococcus	Meningococcus	Total	
More sensitive to X 16-fold			1		1					0					0	More sensitive to X 8-fold
8-fold		1			1					0	1			1	2	4-fold
4-fold		2		2	4	1				1	1	2			3	2-fold
2-fold	4	1			5	2	1			3	3		1		4	Same sensitivity to X and G
Same sensitivity to X and G	6		1		7	5	3	1	1	10	4	2	1	1	8	More sensitive to G 2-fold
More sensitive to G 2-fold					0	2	1			3	1				1	4-fold

* This determination was not made in the case of one strain.

strains to penicillins X and G, and the sensitivities after they had been made resistant. The control cultures which were passed along in broth without penicillin often showed changes in sensitivity to penicillin when tested at the end of the experiment. Usually these were slight, there being a 2- or 4-fold increase or decrease, as compared with the original sensitivity. In the case of 3 of the staphylococcus strains, the sensitivity of the control cultures increased 4- to 18-fold. No explanation can be given for this phenomenon. In every instance, except 1, the resistance of the strains transferred in penicillin was raised above that of the control cultures.

The degree of resistance which could be induced varied greatly from strain to strain and also in the same strain, depending upon which fraction was used. The resistance of some strains was raised higher by means of penicillin X, and that of others was raised higher with the use of penicillin G. When the resistance of a strain had been raised by employing 1 fraction, there was little difference in the sensitivity of that strain to the 2 different fractions. For instance, the sensitivity of 1 staphylococcus strain was 28.6 units per ml. of either penicillin X or penicillin G when its resistance had been raised by means of penicillin X, and 3.6 units per ml. of each penicillin fraction when its resistance had been raised by the employment of penicillin G.

In Table IV we have compared the relative sensitivity of the organisms to the 2 fractions of penicillin before and after the induction of resistance. When the comparison is made on a unit basis, 11 of these strains were more sensitive to penicillin X than to penicillin G before their resistance was raised, and 7 were equally sensitive to the 2 fractions. After the resistance of these strains was raised to penicillin X, only 4 were more sensitive to that fraction than to penicillin G, while 10 were equally sensitive to the 2 fractions, and 3 were more sensitive to penicillin G. On the other hand, after the resistance of the strains had been raised to penicillin G, 9 were more sensitive to penicillin X, 8 were equally resistant to the 2 fractions, and 1 was more sensitive to G than to X.

As has been stated before, the weight of each fraction of penicillin used in testing the strains is a truer method of comparison. When the comparison is made in this way (the extreme right-hand column in Table IV) it is seen that the number of strains possessing greater sensitivity to penicillin X before their resistance was raised, was approximately the same as the number of those possessing greater sensitivity to penicillin G. After their resistance had been raised to penicillin X, 1 was more sensitive to penicillin X, 13 were more sensitive to penicillin G, and 3 were equally sensitive to the 2 fractions; whereas after their resistance had been raised to penicillin G, 5 were more sensitive to penicillin X, 9 to penicillin G and 4 were equally sensitive to the 2 fractions. It is evident from these figures that raising the resistance of these strains to penicillin X caused them to be relatively more sensitive to penicillin G than to penicillin X, whereas raising their resistance to penicillin G left the proportions of those resistant to the 2 fractions about the same as before.

Studies on strains which became resistant in patients under treatment with penicillin

While we were investigating the relative sensitivity of bacteria to penicillin X and penicillin G, we encountered a patient, J. F., with a bacterial endocarditis caused by a *Streptococcus viridans* which developed increasing resistance to penicillin while the patient was under treatment with this antibiotic.

This patient was a 22-year old colored male with a questionable history of syphilis, and no history of rheumatic fever. Three weeks before admission he developed headache, followed by pain and stiffness in the cervical spine and swelling of both feet and ankles. He was acutely ill on admission to the hospital, with a temperature of 102° F, a pulse rate of 112, and a blood pressure of 140 systolic and 60 diastolic. There was a systolic thrill in the 3rd and 4th interspaces to the left of the sternum, and systolic and diastolic murmurs, maximal in the same area and transmitted over the entire precordium. The liver edge was 4 cm. below the right costal margin. The spleen was not felt. Roentgenogram of the chest revealed slight enlargement of the left ventricle and of the pulmonary conus area, and a moderate increase in the pulmonary vascular shadows.

When 6 blood cultures, taken on different days, had grown the *Streptococcus viridans*, as shown in Figure 1, the patient was started on penicillin G by mouth. For the first 24 hours the dose was 100,000 units in amphojel every 4 hours; and for the next 24 hours, 200,000 units every 4 hours. Since this did not seem to be influencing the course of the disease, the dose was changed to 25,000 units of penicillin G intramuscularly every 2 hours. A test of the etiologic organism at this time for sensitivity
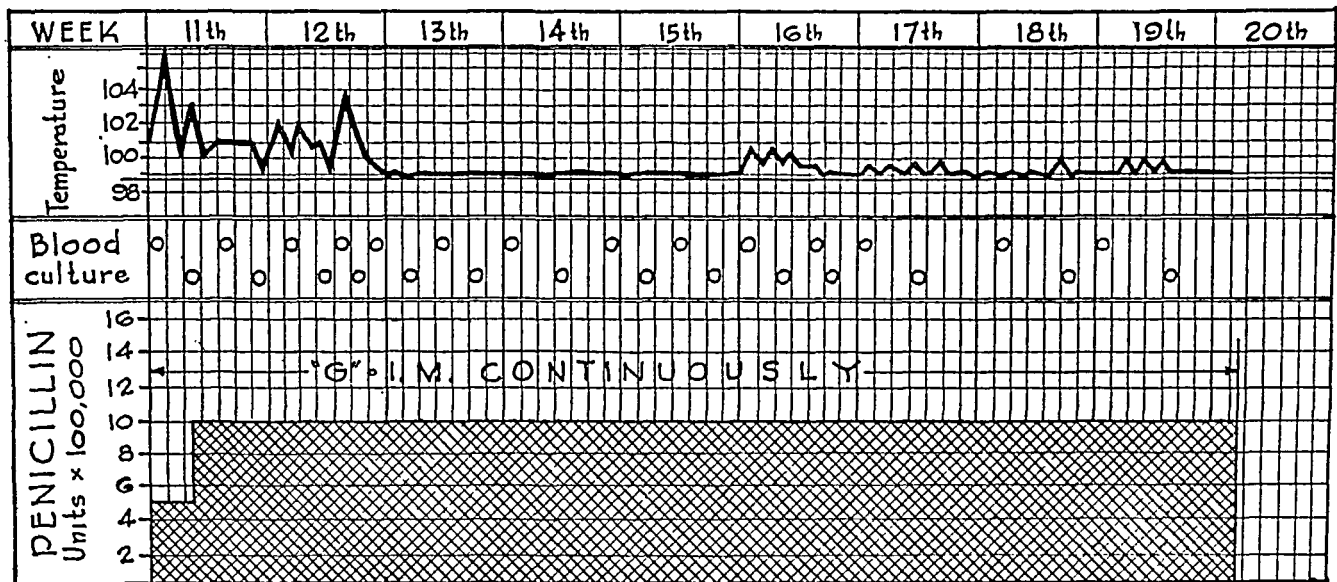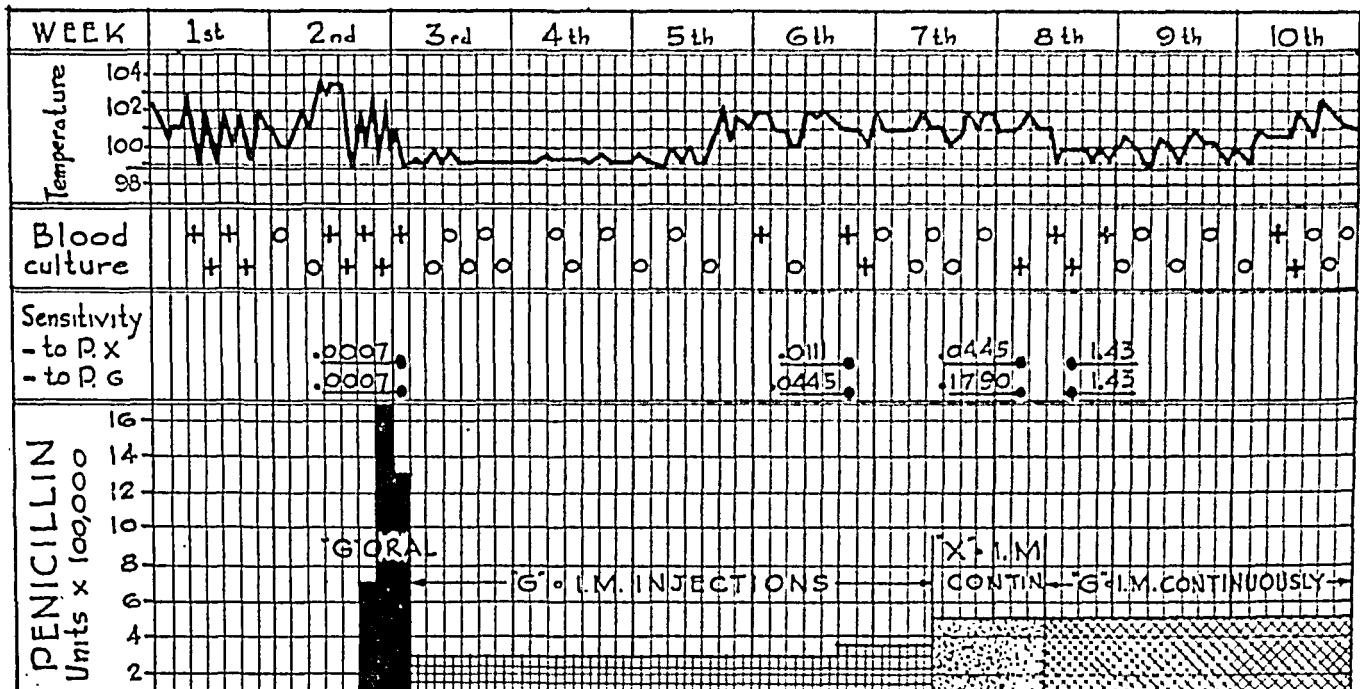
F.J.   C.M. 22   BACTERIAL ENDOCARDITIS

WEEK	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th

Temperature (104, 102, 100, 98)

Blood culture

Sensitivity
– to P. X
– to P. G

.0007   .0007   .011   .0445   .0445   .1790   .1.43   1.43

PENICILLIN Units × 100,000 (16, 14, 12, 10, 8, 6, 4, 2)

'G' ORAL   'G' I.M. INJECTIONS   'X' I.M CONTIN- 'G' I.M. CONTINUOUSLY

WEEK	11th	12th	13th	14th	15th	16th	17th	18th	19th	20th

Temperature (104, 102, 100, 98)

Blood culture

PENICILLIN Units × 100,000 (16, 14, 12, 10, 8, 6, 4, 2)

'G' I.M. CONTINUOUSLY

FIGURE 1.

to penicillin revealed that 0.0007 unit per ml. of either the X or G fraction was sufficient to inhibit its growth.

The temperature fell and the blood cultures became negative on the intermittent intramuscular injections, but after a week fever and bacteremia reappeared. The sensitivity of the organism cultured at this time had risen to 0.01 unit per ml. in the case of penicillin X, and 0.04 unit in the case of penicillin G. Penicillin X was tried in doses of 500,000 units per day by continuous intramuscular infusion without success. Positive blood cultures con-

tinued to make their appearance, and the sensitivity of the organisms reached 0.04 unit per ml. and 0.18 unit per ml. for the X and G fractions, respectively. Due to the scarcity of penicillin X at that time, treatment was resumed with penicillin G in doses of 500,000 units per day by continuous intramuscular infusion. The sensitivity of the organism was again tested and found to be 1.4 units per ml. for both the X and G fractions. As a result of this, the dose was doubled. After a few days on 1,000,000 units per day by intramuscular infusion, the patient im-

TABLE V

Relative sensitivities to penicillin X and penicillin G of a strain of Staphylococcus aureus isolated from a patient with endocarditis

	Sensitivity of organism	
Day of disease	Sensitivity to penicillin X	Sensitivity to penicillin G
	units per ml.	*units per ml.*
15	0.01	0.04
29	0.02	0.09
34	0.09	0.2
36	0.09	0.2
41	0.2	0.4

proved considerably, the temperature fell, and the blood culture became and remained sterile. After 9 weeks of this regime, the patient left the hospital against advice. He was examined 4 months later and found to be free of symptoms and of all evidence of infection.

With the exception of the first few days, when the patient was being treated orally, the concentrations of penicillin in the blood were always higher than the amount of penicillin required to inhibit the growth of the organism *in vitro*. In spite of this, the infection remained active until a dose of 1,000,000 units per day was given.

Another patient, L. M., a 34-year old colored female, had an endocarditis caused by *Staphylococcus aureus*, an organism which became more resistant to penicillin during the course of treatment. When this organism was first cultured from the blood, on the 15th day of the disease, it was sensitive to 0.01 unit per ml. of penicillin X and to 0.04 unit per ml. of penicillin G (Table V). On the 29th day, its resistance to each fraction had increased 2-fold. Organisms cultured from the blood at intervals during the next 12 days showed gradually increasing resistance to both the X and G fractions until 0.2 and 0.4 units per ml. respectively, were required to inhibit the growth of the organisms.

During the period in which these cultures were obtained, the patient was being treated with increasingly larger doses of penicillin G, starting with 15,000 units every 3 hours by intramuscular injection on the 15th day of the disease. Before the organisms were cultured from the blood for the last time (on the 41st day), the patient had been receiving 1,000,000 units per day by continuous intramuscular infusion. She was continued on penicillin treatment for 8 more weeks, during which she became afebrile and symptom-free, and her blood cultures remained negative. She has recently been discharged from the hospital, apparently infection-free.

DISCUSSION

The excellent results obtained with the commonly used G fraction of penicillin do not preclude the possibility that some other fraction may be still more effective. It is necessary, therefore, to determine whether bacteria causing human inf tions are more sensitive to the X fraction than penicillin G, or whether they develop resistar against 1 fraction without developing it agai the other. Upon comparing the relative sensitiv of various bacteria from human sources to appro mately equal amounts of penicillin X and penicil G by weight, we found that slightly more than of the strains were more sensitive to X than to ¼ were equally sensitive to both fractions, a slightly less than ¼ were more sensitive to G th to X. When our figures are compared with Or (4), using the weight of the penicillin fraction a basis of measurement in both instances, the sults are very similar. Libby and Holmberg (compared the sensitivity of a few organisms known weights of penicillin X and G, and fou that the ratio of the number of μg. of penicillin to the number of micrograms of penicillin X quired to inhibit growth of the organisms var from 0.6 to 2.0. It is evident from these 3 vestigations that, while some strains of bacte tested showed considerably more sensitivity penicillin X than to penicillin G, and some shov more sensitivity to G than to X, nevertheless m strains showed very little difference in sensitiv to the 2 fractions.

Since most of the bacteria which are causing man infections at the present time have presuma not yet come in contact with penicillin, it is p sible that great differences in their sensitivi to the various fractions of penicillin will deve only after prolonged contact with 1 of these fr tions. We might expect the organism to beco relatively more resistant to the fraction w which it had been in contact. Actually, as sho in Table III, when a strain's resistance to 1 fr tion was increased, its resistance to the other fr tion increased also. Further evidence is provic by the cases of the 2 patients with bacterial en carditis: the organisms developed resistance both penicillin X and G while the patients w being treated with the G fraction. Flippin and associates (5) reported the development of sistance to penicillin G by the organism of a tient under treatment with this fraction. Bef treatment, 0.025 unit of penicillin G per ml. sufficient to inhibit its growth. Six months la 0.75 unit per ml. was required. At the same ti however, growth of the organism was inhibited

only 0.05 unit per ml. of penicillin X. Treatment was changed from penicillin G to penicillin X, and soon after this the patient improved and apparently recovered completely. Since no determination of sensitivity to penicillin X was made on the organism recovered before penicillin was administered, it is impossible to tell whether the organism developed any resistance to this fraction during the course of treatment. In the case of our patient, with *Streptococcus viridans* endocarditis, the organism was found to develop resistance to the 2 fractions simultaneously, the sensitivity finally reaching 1.43 units per ml. of either fraction. Fortunately, the patient recovered after prolonged treatment with 1,000,000 units of penicillin G per day. The organism in the case of our patient with *Staphylococcus aureus* endocarditis also developed increasingly greater resistance to both the X and G fractions simultaneously.

It has already been pointed out that, following passage of the organisms through penicillin X, resistance to penicillin X was developed to a measurably greater degree than resistance to penicillin G. Similar results were obtained when strains were passed through penicillin G. In every instance, however, there was a simultaneous increase in resistance to both fractions, and this increase was greater than the difference between the fractions.

SUMMARY AND CONCLUSIONS

1. One hundred and one strains of bacteria from human sources were tested for sensitivity to the 2 penicillin fractions X and G. When the weights of the penicillin fractions employed were used as the basis for comparison, 55 strains were from 2 to 16 times more sensitive to penicillin X than to penicillin G, 21 were 2 to 4 times more sensitive to penicillin G, and 25 were equally sensitive to each fraction.

2. Sixteen strains were made resistant to penicillin X and penicillin G by serial passage in media containing increasingly larger amounts of these fractions. At the termination of the experiments, some of the strains whose resistance had been raised to penicillin X had become relatively more

sensitive to penicillin G than to penicillin X. Among the strains made resistant to penicillin G, there was no significant difference between the number which were more sensitive to penicillin X than to penicillin G at the beginning of the experiments, and the number which were more sensitive to penicillin X at the end of the experiments. In every instance when the resistance to 1 fraction was raised, the resistance to the other fraction followed along, to the same or nearly the same extent.

3. The sensitivity of the etiologic organisms to penicillins X and G were studied in a patient with *Streptococcus viridans* endocarditis and in a patient with *Staphylococcus aureus* endocarditis. In each instance, while the patient was receiving penicillin, the organism responsible for the infection developed resistance to both penicillin fractions simultaneously.

4. It is concluded that organisms which cause human infections usually do not show great differences in their relative sensitivity to penicillin G and penicillin X. In most instances, when resistance to 1 of these fractions increases, resistance to the other fraction will also increase.

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THE EFFECT OF INFECTION AND TRAUMA ON THE EXCRETION OF URINARY CORTIN [1]

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Adrenal cortical hyperplasia which is known to occur during infection and after trauma (1, 2) suggests the occurrence of a corresponding increase in the physiological activity of the gland. Scant information is available, however, bearing directly on the functional status of the adrenal cortex during conditions of stress. It would be important to know not only whether periods of stress are accompanied by increased cortical function, but also whether recovery might be influenced by the functional state of the adrenal cortex, and whether such conditions as post-infectious asthenia might be attributable to adrenal exhaustion resulting from extreme stimulation during the active phase of the disease.

Although the excretion of neutral 17-ketosteroids is influenced by adrenal cortical function, the chemical determination of these steroids affords a poor measure of the activity of the gland, inasmuch as the adrenal is not the sole source of the compounds in this fraction. Moreover, information as to the precursors of urinary 17-ketosteroids is far from complete. The one classical precursor, testosterone, has insignificant cortin-like activity.

Browne and coworkers demonstrated the presence of cortin-like activity in the urine of postoperative patients and patients suffering infection or other types of stress, but were not able to detect the material in normal urine (3a and b). Evidence has recently been adduced that the substance is present in the urine of normal men and women who have not been subjected to stress, and that it possesses the physiological properties of cortical hormone when tested by a great variety of methods (4 to 9). Furthermore, the material has been observed to disappear from the urine of monkeys after adrenalectomy, but not after gonadectomy (10), and activity has not been detectable in the urine of patients with Addison's disease (11). It may therefore be assumed with justification that the urinary substance has its origin in the adrenal cortex, and that its rate of excretion should be influenced by variations in cortical function.

The intent of the work herein reported was to determine the urinary cortin output of human subjects in the normal state, during stress, and during recovery from stress. It was considered desirable to employ methods which would allow expression of cortin values in terms of a pure cortical hormone, rather than as arbitrary biological units. These studies are a part of a broader investigation of the physiology and chemistry of active urinary cortical hormones.

METHODS

Preparation of extracts. Urine samples were collected in 48-hour lots, and were preserved with toluene. The pH varied from approximately 5.5 to 6.5. One-fourth volume of ethylene dichloride was added, and after being shaken for 2 minutes in a mechanical shaker, the ethylene dichloride was separated from the urine with a Sharples centrifuge. The urine was extracted twice more with the same volume of ethylene dichloride and the 3 extracts pooled and evaporated *in vacuo* at an internal temperature below 40° C. After twice adding 95 per cent ethanol and evaporating to dryness to remove residual ethylene dichloride, the residue was taken up in 50 ml. chloroform, cooled in an ice bath and washed 3 times with 0.1 N NaOH. Each washing of NaOH was backwashed with 5 ml. of chloroform. All chloroform fractions were pooled and washed with 5 ml. H_2O 4 times, or until free from NaOH. The chloroform was evaporated *in vacuo*, and the residue was taken up in 95 per cent ethanol and stored at −15° C. until used. Under these conditions there appeared to be no loss of activity for at least 4 months.

Methods of assay. Two methods of assay were employed. One method was based upon the protection of adrenalectomized rats to exposure to cold. The other

made use of the glycotropic activity exhibited by certain cortical hormones. Reported elsewhere are details of the methods along with a critical appraisal of their reliability (12, 13).

The cold test is the simplest and most sensitive method now available for the assay of cortin-like substances. A 10 ml. portion of normal urine contains sufficient active material to exert a protective effect on a rat. The response shows a gradation in relation to dose, and may be expressed in the form of a log-dose response curve. The initial straight line portion of the curve is very short, however, and in the case of urine extracts at levels beyond 4 times the minimal effective dose the response declines. The most serious defect of the method is an unexplained variability of sensitivity of test animals from lot to lot. In only about 2 out of 3 trials will a group of rats show sufficient reactivity for satisfactory assay.

The application of the method was as follows: One hundred rats divided into groups of 10 were run simultaneously. One group served as control, 1 received 64 γ of compound A of Kendall (11-dehydrocorticosterone[2]), and

[2] We are indebted to Dr. E. C. Kendall for the greater part of the supply of 11-dehydrocorticosterone used in these studies.
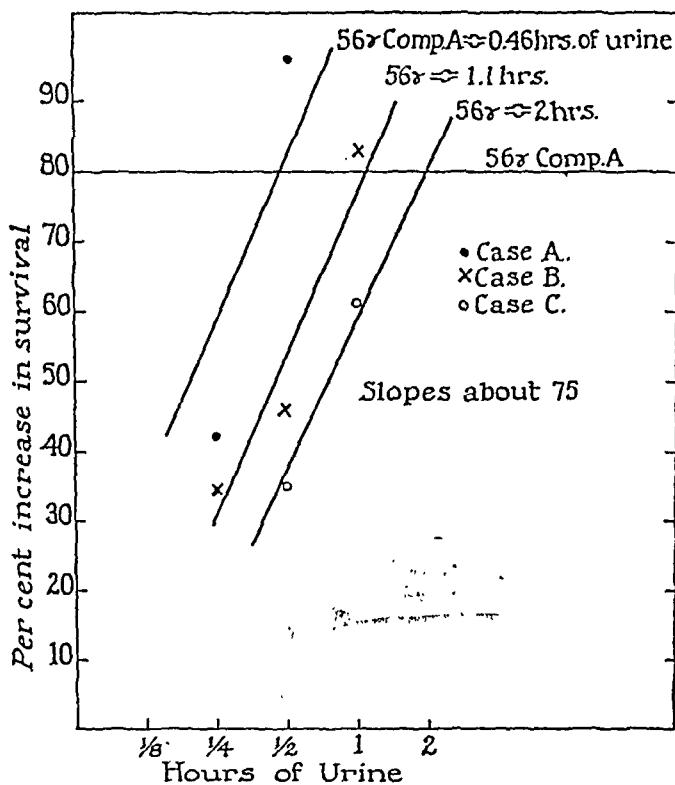
FIG. 1. COLD TEST

Nine groups of 10 animals each were run simultaneously, with 1 group serving as untreated controls, and another receiving 56 γ of compound A. The point is noted at which a line for a urine sample crosses the horizontal line representing the response to compound A. The abscissal value of this point represents the equivalent in hours of urine which is comparable in activity of 56 γ of compound A.

the others received urinary extract in doses equivalent to from ¼ to 1 hour of urine output. Each urine sample was administered at a minimum of 2 different dose levels. If the group receiving compound A survived at least 20 per cent longer than the control group, and if the 2 dose levels of urine extract followed a rising curve, the results were considered satisfactory for calculation of potency (Figure 1). Inasmuch as the sensitivity of various lots of animals is variable, the slope of the response curve is likewise variable, and the error of an assay cannot be predicted in advance. When 2 dose levels of unknown are used, and when sensitivity is maximal, the limits of error are ⅓ as great, or 3 times greater than the observed value with $p = 0.05$. When sensitivity is at the lowest accepted level (20 per cent increase to 64 γ of compound A) these limits are ⅙ and 6. Inasmuch as the slope, furthermore, must be estimated for each assay, it is clear that values which were obtained by the cold test method were rough estimations.

The glycogen test as employed for assay is about ¼ as sensitive as the cold test, and is rather laborious, but is more reproducible and subject to less error. There is a long rising portion of the log-dose response curve without a decline at higher dose levels, and there is no indication of a lack of sensitivity of animals from group to group.

In the use of this assay, 8 to 10 mice received a given dose of urine extract (usually 3 hours of urine equivalent). The mouse livers were pooled and the glycogen content of the pooled sample determined. Potency of an extract was estimated by reference to a standard curve obtained by the administration of graded doses of compound A (11-dehydrocorticosterone) to a series of 193 mice. The usual limits of error of an assay ($p = 0.05$) were calculated to be 3 times higher, or ⅓ as great as the observed value (error factor of 3).

RESULTS

The cortin content of normal urine. A pooled sample of male urine (N–197), when assayed against compound A, gave a value of 0.6 mgm. per liter by the cold test, and 0.4 mgm. by the glycogen test. The error factor of the latter was 1.6 with $p = 0.05$, and 1.9 with $p = 0.01$. The assay curves of compound A and pooled normal urine are illustrated in Figure 2. The details of these comparisons are recorded elsewhere (12, 13).

In Table I are listed assays on 17 normal subjects, ranging in age between 24 and 38 years. Nothing suggestive of a difference between males and females may be discerned from the data. The cold test tended to give higher values than the glycogen test. In the case of the former, the range was between 0.5 and 1.8 mgm. compound A equivalent per day, with an average of 1.1 mgm.; in the case of the latter, the values ranged from

< 0.2 mgm. to 0.8 mgm., and averaged less than 0.4 mgm.

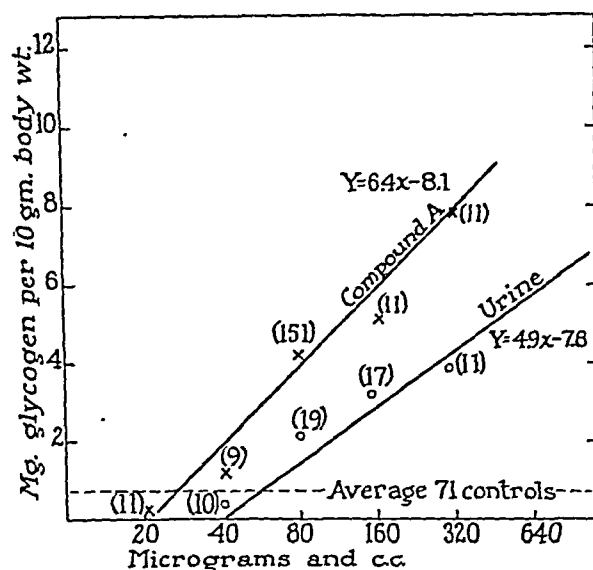It is seen that with both pooled· urine and with

FIG. 2. GLYCOGEN TEST

The regression line on the right represents the response to a sample of pooled normal urine when administered at 4 different dose levels. In the assay of individual samples as described in the text, 1 dose level was used and the dose was expressed as hours of urine. Potency of the urine sample was calculated by noting the point on the line for compound A which gave the same glycogen value as. the sample of urine.

TABLE I

Cortin excretion in normal subjects

Method of assay	Sex	Hours of urine per animal	Cortin excretion compound A-equiv.
			mgm. per day
Cold test	F	½, ½, 1	1.4
	M	¼, ½, 1	0.7
	M	1	0.5
	M	½	1.0
	F	¼	1.5
	M	½, ½, 1	1.8
	M	½	1.0
Liver glycogen test	M	3	<0.3
	M	3	<0.3
	M	2	0.5
	F	2	<0.5
	F	2	0.8
	F	4	0.5
	M	4	0.4
	M	4	<0.2
	F	4	<0.2
	M	2	<0.5
	M	4	0.3
	F	4	<0.2

individual assays the cold test gave higher values than the glycogen test. This difference, if not due to a chance error of the assays, may be due to the presence in the urine of steroids which have relative activities in the 2 tests differing from those of compound A. Desoxycorticosterone, for example, is much less active in its effect on liver glycogen than it is in the cold test.

TABLE II

Cortin excretion as affected by infectious disease

Case	Sex	Age	Diagnosis	Maximum fever	Duration of fever	Treatment	Type of assay	Cortin excretion compound A equiv.					
								Active phase		Early convalescence		Late convalescence	
								Range	Average	Range	Average	Range	Average
					days			*mgm. per day*	*mgm. per day*	*mgm. per day*	*mgm. per day*	*mgm. per day*	*mgm. per day*
H. M.	M	22	Lobar pneumonia	40.8	5	Penicillin	C. T.* L. G.†	0.6 to 3.2 0.5 to 3.1	1.8 1.8	1.4 to 1.6 0.5 to 5.7	1.5 2.3	1.1 to 1.8 0.7 to 0.8	1.5 0.7
G. P.	F	31	Acute enteritis	40.5	3	Symptomatic	C. T.	1.2 to 6.0	3.6	0.6 to 3.0	2.1	1.0	1.0
B. H.	F	19	Acute tonsillitis	39.8	3	Sulfadiazine	L. G.	<0.5 to 3.7	2.1	<0.5 to 0.5	0.5		
M. B.	F	20	Acute pharyngitis	39.2	7	Symptomatic	L. G.	<0.5 to 0.5	0.5	<0.5 to 1.2	0.8		
E. H.	M	31	Typhoid fever	41.0	24	Symptomatic	L. G.	0.7 to 3.4	2.0	0.4 to 0.8	0.7		
J. B.	M	25	Lobar pneumonia	40.2	1	Penicillin	L. G.	0.4	0.4	0.4 to 1.5	0.7		
V. L.	M	19	Fulminating meningococcemia	40.0	1	Penicillin	L. G.	0.6	0.6				

* Cold test.
† Liver glycogen test.
The febrile period was defined as the active phase of the disease; the first week after fever subsided was considered to be the phase of early convalescence; and the second week, late convalescence.

Urinary cortin as influenced by infection. Seven patients with infectious disease were studied. The findings are tabulated in Table II. Five of the patients showed increases in excretion during the febrile, or early post-febrile stages, which were higher than levels encountered in normal subjects. The 2 patients which showed no augmentation in output were ill for only 1 day. One (J. B.) was a very early case of lobar pneumonia promptly aborted with penicillin, and the other (V. L.) was a patient with fulminating meningococcemia who died within 24 hours after onset. The latter case is of some interest inasmuch as the patient was admitted in shock and fulfilled all of the clinical qualifications of the Waterhouse-Friderichsen syndrome. The presence of a normal level of urinary cortin argues that the shock was not due to adrenal insufficiency. An autopsy could not be obtained.

During the period of early convalescence, the 4 patients who had excreted increased amounts of cortin during the febrile stage showed a decline to normal or slightly hypernormal levels. In late convalescence the 2 patients who were followed showed normal levels of excretion. In no case was there any consistent decline to subnormal levels during the period of convalescence. Patients B. H. and E. H. experienced post-infectious asthenia during the early convalescent period, but their cortin excretion during this time did not differ from that of the other patients.

Cortin excretion as affected by surgery. Two patients undergoing herniorrhaphy were studied (Table III). Assays were done on patient C. D. for a 48-hour period at home before admission to the hospital, and for another 48 hours pre-operatively while he was confined to bed. An ambulatory control period was not included in the case

of patient J. W. In both cases there was a distinct rise in excretion during the first 2 to 4 days post-operatively. Patient J. W. showed an increase more than 10-fold above his pre-operative level. After 6 days the levels in both cases had returned to normal. There were no post-operative complications. It should be noted that patient C. D. received a local anesthetic, and that the response in this instance may therefore be attributed to the surgical procedure *per se*.

Cortin excretion after body burns. This group included 3 patients (Table IV). The 1 patient (M. D.) who recovered was studied over a fairly long period of convalescence. There was an unmistakable rise in cortin excretion during the first 8 days after the burn. By the end of 12 days the elevation was less marked, and beyond 12 days the values had declined to within the range of normal. Patient C. D. showed a moderate elevation shortly before his death on the ninth day. Patient A. T., who also died, did not excrete quantities of cortin in excess of the values normally encountered.

The results herein reported confirm and extend the original findings of Weil and Browne. Venning and Browne (14), furthermore, have recently demonstrated a rise in urinary cortin during stress when assays were performed by a glycogen method. Our most formidable hurdle has been the development of quantitative assay methods possessing sufficient simplicity and reliability to be applied to clinical cases. Methods which are now available still leave much to be desired. It is possible, however, that further refinement of the glycogen procedure will render it reasonably satisfactory for this purpose. Talbot and coworkers (15) have recently devised a chemical procedure for the assay of adrenal cor-

TABLE III

Cortin excretion after herniorrhaphy

Case	Sex	Age	Operation	Anesthetic	Type of Assay	Cortin excretion compound A equiv.							
						Preoperative		Postoperative day					
						Ambulatory	In bed	1 to 2	3 to 4	5 to 6	7 to 8	9 to 10	11 to 12
C. D.	M	35	Bilateral herniorrhaphy	Local	C. T.	1.6	0.5	2.3	3.0	1.9	0.9	1.1	0.6
J. W.	M	26	Rt. inguinal herniorrhaphy	Ether	L. G.		<0.5	4.9		0.5		0.8	

TABLE IV

Cortin excretion in the presence of body burns

Case	Sex	Age	Percent body area	Type of assay	Cortin excretion compound A equiv. Days after burn									
					1 to 4	5 to 8	9 to 12	13 to 16	17 to 20	21 to 24	25 to 28	29 to 32	33 to 36	90
								mgm. per day						
C. D.	M	46	50	C. T.		2.7	Died							
A. T.	M	48	30	L. G.	0.6	0.8	<0.5	Died						
M. D.	F	26	50	L. G.	3.5	3.7	1.4		0.7		0.7		0.5	<0.3

We are indebted to Dr. W. E. Abbott of the Department of Surgery, Wayne University, for the urine specimens on A. T. and M. D.

tical steroids in urine. The advantages of a chemical method are obvious, but interpretations of results must be made with caution until the compounds which take part in the reaction have been identified, or until it has been shown that values obtained by the chemical method correlate with those derived by bioassay.

It is apparent that the amount of cortin-like material present in normal human urine is fairly large. Without doubt the values which were obtained are minimal estimates. The urine was extracted with solvent only 3 times. Further extraction both before or after hydrolysis has been shown to yield additional quantities of active material. It is of interest to note that the chemical estimate of corticosteroid excretion obtained by Talbot in normal subjects is not far from the values we have secured by bioassay.

It is reasonable to assume that the increased cortin output during stress is a reflection of increased cortical hormone production. Other possible interpretations are a decreased tissue affinity for the hormone, an increase in the rate of renal excretion, or a decreased inactivation. It is unlikely that decreased tissue affinity or increased excretion could be responsible for the augmented output. The possibility of decreased inactivation could be more easily defended, however.

Adrenal cortical hypersecretion would fit very nicely into the pattern of physiological readjustment which is known to result from the stimulus of stress. Selye was the first to emphasize that the organism reacts in identical fashion to a variety of damaging stimuli (16). He emphasized, moreover, that the adrenal cortex was probably of prime importance in the facilitation of adjustment. In order to simplify discussion, some of the possible physiological and clinical implications of cortical hyperfunction during stress are outlined in tabular form (Table V). Albright (17) has recently discussed a number of these mechanisms.

If one assumes that increased cortical secretion during stress is beneficial to the organism, it follows that any failure in this function should influence the course of recovery. If it can be shown that in some cases of infection or other stress a low output of cortin is associated with a higher mortality or a delay in recovery, it would be justifiable to administer cortical hormone to such patients. Such a correlation has as yet not been made, however, and evidence does not exist that in the absence of specific adrenal disease hypo-

TABLE V

Possible functions of adrenal cortical hormone during stress

Observed changes	Physiological aspects	Beneficial aspect	Possible disadvantage
Negative nitrogen balance Hyperglycemia	Increased gluconeogenesis	Source of endogenous carbohydrate	Wastage of protoplasm in prolonged disease. Decreased carbohydrate tolerance in presence of diabetes
Tendency to retain salt and water	Increased reabsorption of sodium by kidney	Support of plasma volume	Hemodilution and edema after administration of large quantities of saline
Lysis of lymphoid tissue	Release of antibodies	Defense against infection	

function of the gland is a factor in recovery from stress.

SUMMARY AND CONCLUSIONS

1. In normal subjects the daily excretion of urinary cortin averaged about 1.1 mgm. of compound A-equivalent per day when assayed biologically by a cold protection test, and < 0.4 mgm. per day by a glycogen deposition test. There was no obvious difference in the output of male and female subjects.

2. In the presence of infectious disease, after body burns, and during the first 2 days after herniorrhaphy, the output of urinary cortin was usually increased above the control levels. The rise in some instances was as high as 10-fold.

3. During recovery from stress there was no abnormal depression in output which one would expect in the presence of adrenal exhaustion.

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PARENTERAL NUTRITION. II. THE UTILIZATION OF EMUL-SIFIED FAT GIVEN INTRAVENOUSLY [1]

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The intravenous administration of various fat emulsions to dogs has been studied in this laboratory (1). Of the emulsions used, one consisting of 15 per cent refined coconut oil stabilized with a purified preparation of soybean phosphatides was found to be well tolerated, and produced only minor pathologic effects after daily infusions for periods of 1 month. In these experiments, amounts of the emulsified fat were used sufficient to give 15 or 16 calories per kgm. of body weight per day. The use of such an emulsion for nutritive purposes would depend on whether or not fat administered in this manner can be efficiently utilized as a source of energy. A study of this problem forms the basis of the present paper.

The utilization of fat given intravenously has been studied by several investigators. Nomura (2) found no decrease in the respiratory quotient of dogs following the infusion of an olive oil-cod liver oil emulsion stabilized with egg phosphatides. This was also found in dogs which had fasted 4 to 8 days previous to the infusion, although the initial respiratory quotients were much lower in this series. Baba (3) fed dogs a meat diet with thyroxin to produce energy deficiency and weight loss. Infusion of this same olive oil-cod liver oil emulsion produced slight decreases in the non-protein respiratory quotient of the magnitude of from 0.75, to 0.70 or 0.71. In depancreatized dogs Baba (4) observed marked decreases in the non-protein respiratory quotient ranging from between 0.69 and 0.71 to the very low values of between 0.59 and 0.64 Partially depancreatized dogs showed a smaller decrease in the respiratory quotient. Murlin and Riche (5) found a decrease in the respiratory quotient of from 0.79 to 0.72, and 0.85 to 0.73, several hours after infusion of a 3 per cent

lard emulsion into dogs. Gordon and Levine (6) observed decreases in the respiratory quotient of from 0.03 to 0.07 following infusion of an olive oil emulsion stabilized with egg phosphatides in a healthy infant, starting with initial respiratory quotients between 0.9 and 1.0. At a control respiratory quotient of 0.8, no significant lowering was produced by infusions of the fat emulsion. No decreases in the respiratory quotient were obtained after infusion of this emulsion into a marasmic infant. In general, it may be concluded that these studies on the effect of intravenous fat emulsions on the respiratory quotient indicate that the fat is utilized, but the evidence is equivocal since the changes are small. It is unfortunate that the respiratory quotient of starvation approaches that of fat metabolism, and thus reduces the value of this criterion of utilization under the conditions in which it is desirable to use fat intravenously: namely, in serious energy deficiency. More definite evidence of utilization should be obtained by other methods of study.

Narat (7) studied starvation survival in 2 pairs of dogs, 1 of each pair receiving by infusion a small amount of an olive oil emulsion stabilized with egg phosphatides. The control dogs died on the 36th and 31st day of starvation, while those receiving the fat infusions survived through the 46th and 45th day respectively. Weight losses were slightly less in the infused dogs. The author concluded from these differences that the fat was utilized. Dunham and Brunschwig (8) injected lard and olive oil emulsions, stabilized with egg phosphatides and "Demal," into 11 dogs receiving an oral food intake furnishing 60 calories per kgm. per day. Urinary nitrogen excretions were not lowered, as was the case with 5 per cent glucose injections. There was no tendency of the depot fat to be displaced toward that of the infused fat with respect to iodine number, saponification number, or melting point. From these results, the authors

concluded that the intravenously administered fat was not utilized.

Since these studies on utilization of the fat have given controversial or at least inconclusive results, it was thought desirable to investigate this matter further. We have used as criteria of utilization, weight maintenance and nitrogen retention on low oral caloric intakes, and also the disappearance of the infused fat from the animal tissues during the infusion period.

EXPERIMENTAL

Four adult mongrel dogs were used in this study. The emulsified fat was infused into the leg veins from a Murphy Drip bottle as previously described (1). Two emulsions were used in the experiments: Emulsion 11M (1) and Emulsion 11MC, identical with Emulsion 11M,

TABLE I

Composition of fat emulsions (11MC and 11M) for intravenous administration

	grams per liter
Water	800
Refined coconut oil	150
Purified soybean phosphatides	27
Na₂HPO₄	6.0
Cholesterol*	4.2

pH of emulsions equals 7.6

* Omitted in Emulsion 11M.

but containing 2.8 per cent cholesterol [3] dissolved in the coconut oil (0.42 per cent of the emulsion). This latter emulsion appeared to be more stable to autoclaving and standing. The composition of Emulsion 11MC is given in Table I, and its preparation and properties are described in a previous paper (1).

[3] Eastman Kodak Company (from the spinal cords of cattle).

TABLE II

Composition of purified rations

	Ration 1 per 100 grams	Ration 2 per 100 grams
Sucrose		68 grams
Dextrose	55 grams	
Powdered "Amigen"	16 grams	
Casein (SMA, vitamin-free)		24 grams
Salt mixture*	4 grams	4 grams
Coconut oil	25 grams	
Cod liver oil		2 grams
Haliver oil	50 mgm.	
Liver Concentrate Powder 1-20 (Wilson's)		2 grams
Choline chloride	250 mgm.	250 mgm.
Thiamine and riboflavin, each	0.5 mgm.	0.5 mgm.
Pyridoxine hydrochloride	0.25 mgm.	0.25 mgm.
Calcium pantothenate	2.5 mgm.	2.5 mgm.
Nicotinamide	12.5 mgm.	12.5 mgm.
Mixed tocopherols	10.0 mgm.	10.0 mgm.

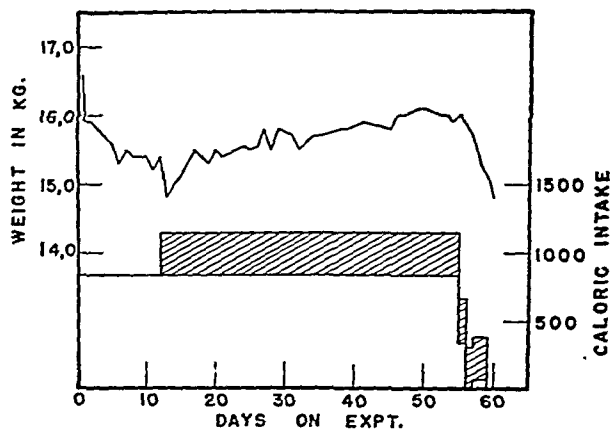* J. Biol. Chem. 1941, 138, 459.
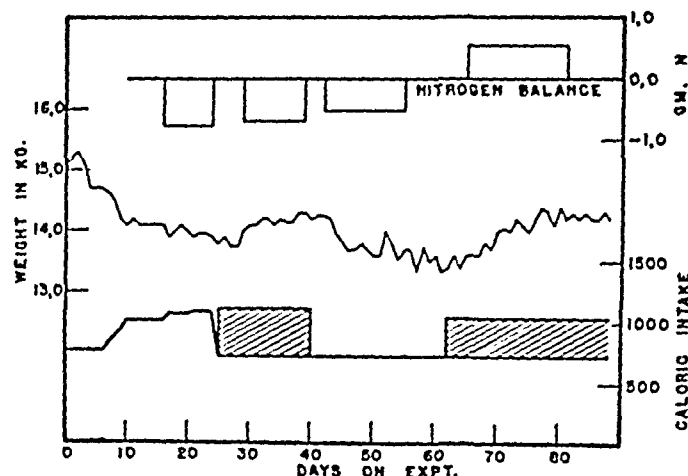
FIG. 1. EXPERIMENT 1. THE EFFECT OF INTRAVENOUS ADMINISTRATION OF FAT EMULSION 11MC ON NITROGEN BALANCE AND BODY WEIGHT OF THE ADULT DOG

Cross hatched areas represent periods of fat infusion.

Experiment 1. A 15.1 kgm. female dog was fed Ration 1, the composition of which is listed in Table II. The ration was calculated to furnish approximately 509 calories per 100 grams. It was supplemented daily with 5 grams of Wilson's Liver Concentrate Powder 1-20, and 9 grams of Cellu Flour, which were added and mixed after each day's ration was weighed out. The ration was fed at varying amounts until an intake just sufficient to maintain weight was obtained (78 calories per kgm., Figure 1). At this point, the amount of coconut oil in the ration was reduced from 25 per cent to 5 per cent, and an amount of the ration given to equal exactly the previous intake of all the other nutrients except fat. The difference in oral intake of coconut oil was then fully made up by the intravenous administration of 290 ml. daily of Emulsion 11MC. This amount of emulsion furnished approximately 390 calories, or 28 calories per kgm. This procedure was continued for 16 days, during which time the weight of the dog was maintained; in fact, there was a slight increase in weight, indicating utilization of the fat administered intravenously, and indicating, also, that the caloric intake was slightly above maintenance. The intravenous administration of fat was then discontinued, and the dog was fed the same amount of the low fat diet for 22 days. This was accompanied by a weight loss, but the nitrogen balance was not significantly different from that observed in the periods before and during the fat infusions. The data on nitrogen balance in this experiment, and in the other experiments reported in this paper, represent an average for the period represented in the diagram, and are determined from combined urine and fecal nitrogen determinations. After this period on the low fat diet, infusion of fat was again started using 239 ml. of Emulsion 11MC per day, which provided 323 calories, or 24 calories per kgm. This produced a striking increase in weight and nitrogen retention, as shown in Figure 1. Thus, it would appear that the fat administered intravenously was definitely utilized. Infusion of the fat emulsion was continued for 27 days, at which time the animal

refused food. At this time (89th experimental day) the blood plasma total cholesterol had reached 476 mgm. per cent, of which 258 mgm. per cent was esterified. Liver function was markedly impaired as measured by poor bromsulfalein elimination, 43 γ of dye per ml. of plasma after 8 minutes, as compared with the normal range of 5 to 20 γ of dye. Prothrombin time (11 seconds) and plasma alkaline phosphatase (126 γ phosphorus liberated per ml. of plasma in 24 hours) were normal. These methods for determining liver function in the dog have been described elsewhere (9). The fat infusions were discontinued and the dog was given milk for 10 days until it would eat the experimental ration. At this time the plasma total cholesterol and esterified cholesterol had dropped to 268 and 134 mgm. per cent respectively. Liver function was almost normal, as measured by brom-sulfalein elimination (20 γ of dye per ml. of plasma) and the dog seemed normal in every respect. The dog was fed *ad libitum* the purified ration with 25 per cent fat, and gained steadily. In 6 weeks, it weighed 18.4 kgm., and was then given commercial dog ration *ad libitum*. After 5 months, the dog was sacrified and a post mortem per-formed. Gross examination revealed no apparent pa-thology. Microscopic examination showed granulomatous nodular lesions in the lungs as previously described (1), but they were less numerous, and were not as large or as prominent as in the previous animals. Foreign body giant cells were less numerous. There was a marked in-crease in scar tissue in the nodules. Hemosiderin was present and was both intra- and extracellular. Small scars were occasionally found which were suggestive of the previous presence of nodules. In the spleen, the granu-lomatous lesions were rare; hemosiderin was present, but in less quantity. In the liver, there was still considerable fat in Kupfer cells of the sinusoids; hemosiderin was pres-ent, but again in lesser amounts; and there was no fatty infiltration of the liver cells.

Experiment 2. A 16.6 kgm. male dog was fed Ration 2 (Table II). The ration was calculated to furnish ap-proximately 386 calories per 100 grams. Throughout the experiment the dog received 215 grams of this ration daily, furnishing approximately 830 calories. Nine grams of Cellu Flour were mixed with the ration each day. After 12 days on this ration, during which time the dog lost 1.2 kgm. of weight, daily infusions of Emulsion 11MC were commenced (Figure 2). A total of 47 infusions were given in 48 days, each infusion averaged 239 ml., and the average time of infusion was 166 minutes. These infusions of fat furnished an additional 316 calories per day (approximately 20 calories per kgm.) and during this period there was a gradual gain in weight. Determinations of the total fecal lipid excretion were made before and after the fat infusions were given. The feces excreted in a number of consecutive days were pooled, dried, and weighed, and a sample extracted with chloroform in a Soxhlet extractor for 16 hours. A 5-day sample of feces, collected before the fat infusions were started, had an average daily dry weight of 18.2 grams and of 2.58 grams of chloroform extractable material. After the fat infusions were instituted, the dry weight and chloroform

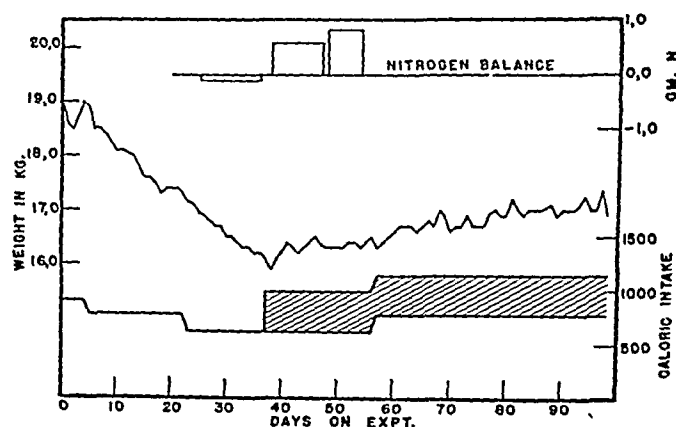FIG. 2. EXPERIMENT 2. THE EFFECT OF INTRAVENOUS AMINISTRATION OF FAT EMULSION 11MC ON BODY WEIGHT OF THE ADULT DOG

Cross hatched areas represent periods of fat infusion.

extractable material of the feces averaged 17.7 grams and 1.57 grams daily for an 8-day collection period, and 23.0 grams and 2.1 grams for another collection period of 7 days. Hence, it appears that none of the fat adminis-tered intravenously was excreted through the intestinal tract. Beginning on the 55th experimental day (44th in-fusion day) the dog suddenly refused his food, and this produced a marked drop in weight (Figure 2). On the 60th experimental day, the total cholesterol and esteri-fied cholesterol of the blood plasma were 432 and 218 mgm. per cent respectively, and the plasma alkaline phosphatase was slightly elevated (462 γ phosphorus liberated per ml. plasma in 24 hours). The blood hemoglobin was 12.5 grams per cent, the hematocrit was 41.3 per cent, and the plasma protein (Kjeldahl) 4.4 grams per cent. These figures may be compared with representative values in our "laboratory dogs" of 12 to 15 grams per cent for hemo-globin, 38 to 48 per cent for hematocrit, and 4.5 to 6 for plasma protein. The dog was sacrificed for post-mortem examination and complete carcass analysis for fat.

Microscopic examination of the organs demonstrated lesions similar to those previously described (1). Small granulomatous lesions were present in the lungs and spleen, and there was evidence of phagocytosis of fat by cells of the reticulo-endothelial system of the liver and spleen. The content of chloroform extractable material in the liver was found to be 42.3 per cent on the dry weight basis, as compared with normal findings of 10 to 20 per cent; however, the other visceral organs were all found to be in the normal range with regard to chloroform ex-tractable material. It is likely that most of this increase in fat in the liver is due to the presence of phagocytized fat in the macrophages of the liver sinusoids. There was no fatty infiltration of the liver. The gall bladder bile was viscous and seemed inspissated, although the total solids were only 25.9 per cent, which is in the normal range for the dog (10). The bile, however, contained 1120 mgm. per cent cholesterol, which is over 30 times the normal amount for the dog (11).

The fat content of the entire dog was determined in the following manner: The visceral organs including the spleen, liver, lungs, pancreas, kidneys, testes, stomach, esophagus, and washed intestines were weighed, and small aliquots of each dried in an oven at 60° C., ground in a mortar and extracted with chloroform in a Soxhlet extractor for 13 hours. The total lipid content of each organ could then be calculated. The skin was removed from the carcass and weighed, and 200 gram aliquot samples selected, cut into small pieces and dried in an oven at 60° C. The dried skin was then refluxed successively with chloroform, and the chloroform extracts freed from chloroform by distilling off the solvent (hot water bath) first at atmospheric pressure, and finally under reduced pressure to constant weight. The chloroform extractable material obtained in this fashion was then weighed, and the total for the skin calculated. Fat depots were dried in the oven and extracted with chloroform in the same manner. The muscle was stripped from the bones, weighed, and ground in a meat grinder. Suitable aliquots of this were dried and extracted as with the other tissues. An aliquot of the bones (amounting to about ⅙ of the total skeleton) was demineralized in a jar of 2 N HCL. The demineralized bones were then cut into small pieces and dried in the oven. The fat was removed from the black, tarry residue by chloroform extraction. The total fat obtained in these fractions is given in Table III. The saponification numbers and iodine numbers of these fractions were determined by the methods of analysis of the Association of Official Agricultural Chemists (12).

Experiment 3. A 19.1 kgm. male dog was fed Ration 2 (Table II) with 7 to 10 grams of Cellu Flour mixed in with the daily ration. *Ad libitum* feeding was permitted for the first 5 days; from the 6th to the 23rd day the daily ration was restricted to 200 grams (772 calories); and from the 24th to the 57th day, it was further restricted to 160 grams (618 calories). Beginning with the 58th day, and continuing to the end of the experiment, 200 grams of the ration were fed. At both levels of caloric

TABLE III

Fat analyses for experiments 2 and 3

Experiment 2

Tissue fraction	Total chloroform extractable material	Saponification number	Iodine number	Calculated maximum coconut oil present
	grams			*grams*
I Depot fat	943	204.5	72.0	198
II Skin	706	206.5	71.6	176
III Muscle and brain	665	211.0	73.3	196
IV Bones	802	212.5	68.1	255
V Visceral organs	136			136
Total	3,252			961

Total coconut oil infused: 1,685 grams.
Minimum total coconut oil unaccounted for and presumably metabolized: 724 grams. (Coconut oil saponification number, 263.5; iodine number, 12.3).

Experiment 3

Tissue fraction	Total chloroform extractable material	Saponification number	Iodine number	Calculated maximum coconut oil present
	grams			*grams*
I Depot fat	802	217.4	57.9	328
II Skin	393	207.9	63.1	107
III Muscle	558	210.3	62.7	171
IV Bones	404	214.3	66.4	147
V Visceral organs and brain	140			140
Total	2,297			893

Total coconut oil infused: 2,455 grams.
Minimum total coconut oil unaccounted for and presumably metabolized: 1,562 grams. (Coconut oil saponification number, 258.4; iodine number, 11.5).

intake the dog lost weight, and by the 38th day had lost 3.0 kgm. (Figure 3). At this time, daily infusions of Emulsion 11M were started and given for 60 of the next 61 days. An average of 273 ml. of emulsion was infused daily during a period of 163 minutes. The daily caloric intake contributed by the intravenous fat was approximately 368 calories, or 22 calories per kgm. The infusions produced an immediate cessation of weight loss and a gradual recovery of lost weight. By the end of the experiment, over 1 kgm. of body weight had been restored. As shown in Figure 3, the nitrogen balance changed from slight negative balance to nitrogen retention when the additional calories were provided by infusion of fat. As in Experiment 2, determinations of total lipids in the feces were made before and after the fat infusions were started. A 7-day sample of feces, collected before the fat infusions were given, averaged, on a daily basis, 20.6 grams dry weight and 1.12 grams of chloroform extractable material. A 10-day sample of feces prior to giving the fat infusions averaged 20.2 grams dry matter per day and 1.40 grams of chloroform extractable material. These 2
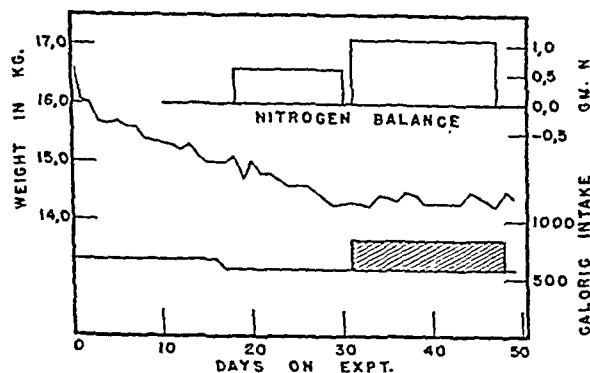

FIG. 3. EXPERIMENT 3. THE EFFECT OF INTRAVENOUS ADMINISTRATION OF FAT EMULSION 11M ON NITROGEN BALANCE AND BODY WEIGHT OF THE ADULT DOG

Cross hatched area represents period of fat infusion.

samples represented the periods of 200 grams and 160 grams daily food intake. After the fat infusions were started, a 10-day sample of feces averaged 17.9 grams dry weight and 1.41 grams chloroform extractable material daily.

The infusions were stopped on the 99th experimental day, at which time the hemoglobin was 10.6 grams per cent, the hematocrit 34 per cent, and the total plasma proteins 5.5 grams per cent. The hemoglobin and hematocrit had decreased from 14.4 and 42 grams per cent respectively, values observed on the 38th experimental day, just before the infusions were started. Liver function was only slightly impaired as measured by bromsulfalein elimination, but normal as judged by prothrombin time (9.5 seconds), cholesterol and cholesterol ester levels of the plasma (288 and 214 mgm. per cent). At this time the dog was sacrificed for post-mortem examination and complete analysis for fat.

Gross examination at autopsy revealed no significant changes, although histologic examination revealed the same general picture observed with the dog of Experiment 2. The liver showed somewhat more fat in the reticulo-endothelial cells, and contained 47.1 per cent chloroform extractable material on a dry weight basis. Again there was no replacement of liver cells by fat. The small granulomatous lesions in the lungs appeared to be more numerous than in previous experiments.

The complete analysis of the animal for fat was achieved by the same general procedure as described in Experiment 2, but all of the bones and skin were extracted rather than aliquots of these tissues, as was done in Experiment 2. Also the brain was extracted separately, whereas in Experiment 2 it was not extracted, but included in the weight of the muscle fraction. The lipid content of the tissues, together with saponification and iodine numbers, are given in Table III.

Experiment 4. A 16.6 kgm. female dog was given the same ration used in Experiments 2 and 3 (Ration 2, Table II) with 7 to 10 grams of Cellu Flour mixed in with the daily ration. From the 1st to the 17th day, 170 grams of the ration were fed daily; and from the 18th

through the 48th day, 150 grams. These 2 levels of intake furnished 656 and 579 calories per day, neither of which was adequate to maintain weight, and by the 32nd day the dog had lost 2.3 kgm. (Figure 4). Daily infusions of Emulsion 11M were started at this time, and 15 infusions, each averaging 225 ml., were given in the next 18 days. The average time of infusion was 138 minutes, and the daily caloric intake from the intravenous fat was 253 calories, or approximately 17 calories per kgm. As observed in Figure 4, the fat infusions produced a maintenance of weight and an increase in nitrogen retention.

DISCUSSION

The improvement in weight and nitrogen retention produced by infusion of the fat emulsions in these experiments, indicates that the fat is utilized by the dog for energy. The ability of the animal to metabolize this fat is demonstrated by Experiments 2 and 3, in which the lipid material of the entire animal was actually isolated, and found to resemble mammalian fat more than coconut oil, insofar as average length of carbon chain (as indicated by saponification number) and degree of unsaturation (as determined by iodine number) are concerned. If we accept a range of 193 to 198 as the saponification number for mammalian (sheep and beef) fat (13), it is apparent that the carbon chain length averages between 16 and 18, for tripalmitin has a carbon chain of 16 and a saponification number of 209, and tristearin a carbon chain of 18 and a saponification number of 189. Dunham and Brunschwig (8) found an average initial saponification number of 206, with a range of 189 to 234, for fat from 13 dogs. Assuming that the minimum saponification number of the fat of the dogs in Experiments 2 and 3 prior to infusion is that of tristearin, then the maximum coconut oil content of the lipids in the tissues of the dog in Experiment 2 was 0.96 kgm.; and in Experiment 3, 0.89 kgm. (Saponification number of coconut oil used in Experiment 2 was 258.4; of oil used in Experiment 3, 263.5. Table III). These values are substantially below the 1.68 and 2.45 kgm. of coconut oil actually infused into the dogs. Since none of the infused fat was lost by urine or feces, it seems clear that this unaccounted for coconut oil has been metabolized, at least to a degree to which it is no longer recognizable as such. The possibility of error or artifact entering into such an experiment must be considered. There may have been errors in sampling, but these could

FIG. 4. EXPERIMENT 4. THE EFFECT OF INTRAVENOUS ADMINISTRATION OF FAT EMULSION 11M ON NITROGEN BALANCE AND BODY WEIGHT OF THE ADULT DOG

Cross hatched area represents period of fat infusion.

hardly be of such magnitude as to account for the difference in these values. Auto-oxidation of the fats must have been kept to a bare minimum, since the mass of fat isolated from both animals was so surprisingly high. In the case of the dog in Experiment 2, the total fat amounted to 22 per cent of the body weight of the animal; and in Experiment 3, to 14 per cent. We have found only 1 reference in the literature on the total lipid content of the dog (14). This paper dealt entirely with young puppies, and gives a figure of 8.5 per cent of body weight for a 100-day-old pup. The total lipid of the rat varies from 16.2 to 23.3 per cent of the body weight in animals 15 weeks old (15) and it is likely that higher figures might be obtained in older animals. It should be pointed out that in all of the fractions except 1, the great majority of lipid is neutral fat. The exception to this is the lipids of the visceral organs which are high in phospholipid, and for this reason saponification numbers of these extracts were not determined. The total lipid in all of the visceral organs is small, however, as compared with the rest of the body fats, and may be either disregarded or considered as pure coconut oil without influencing the conclusions in either experiment. The phospholipids in the extracts of muscle and bone would be expected to lead to a slight error in the saponification number. It should be pointed out also that much of the lipid determined in these 2 fractions actually belongs under "depot fat," but was not separated as such as a matter of convenience.

Utilization of the infused fat is also suggested by a consideration of the iodine numbers of the fat from the dogs in Experiments 2 and 3. If the iodine numbers of the dog fat prior to infusion may be assumed to lie in the range of 48 to 79, as in the case of sheep and beef fats (13), very little coconut oil (iodine number 11.5 to 12.3) could be present in the fats obtained from the dogs of Experiments 2 and 3, certainly far less than the amounts actually infused (Table III). The reasoning with iodine numbers, however, is hampered by the fact that their values may go well above 100 for dog fat, depending on the ration fed, and other factors. Dunham and Brunschwig (8) found an average iodine number of 95 (range, 69 to 119) in the body fat of 13 dogs reported in their studies. Utilization of the infused fat is indicated by the iodine numbers of the body fats in both experiments, but is actually demonstrated only in the case of Experiment 3.

The experimental results reported in this paper point to the conclusion that the infused coconut oil has been utilized for energy. This is contrary to the conclusions reached by Dunham and Brunschwig (8). They observed no tendency toward nitrogen retention during infusions of their emulsions, in dogs receiving an oral intake of 60 calories per kgm. per day. Their emulsions were toxic, since 9 out of the 24 dogs used in the study died during the infusion periods. It was also noted that the emulsions were hemolytic, and that all of the dogs showed some degree of anemia after the infusions. Nitrogen excretion would be expected to be greater in any toxic condition, and is greater during hemolysis. Phenylhydrazine anemia in dogs is accompanied by increased nitrogen excretion (16) and this is also observed in pernicious anemia in relapse. It is conceivable, therefore, that these factors would tend to hide any nitrogen economy provided for by energy obtained from infused fat. Dunham and Brunschwig (8) also observed that the characteristics of the body fats of the infused dogs, as determined by saponification number, iodine number, and melting point, were not markedly displaced toward those of the infused fats. This is in agreement with our own observations, but they concluded from this that the fat was not utilized. As we have mentioned, failure to account in any way for the infused fat should be good evidence for its metabolism. Failure to metabolize or excrete the fat would result in its deposition somewhere in the tissues, which would result in a displacement of the characteristics of the body fat toward that of the infused fat.

The partial impairment of liver function, and the extensive accumulation of fat in the reticulo-endothelial cells of the liver, observed in the dogs of the first 3 experiments, are interesting in view of our failure to observe these changes in previous infusion experiments with Emulsion 11M (1). It should be pointed out, however, that in the earlier experiments lower intakes of fat per kgm. were used, and also for a shorter experimental period. The addition of cholesterol to the coconut oil (Emulsion 11MC, used in Experiments 1 and 2) appeared to hasten the appearance of liver dysfunction. Determination of sterol excretion in

the feces of the dog of Experiment 2 was carried out on the chloroform extracts of the dried feces obtained for the determinations of the total lipids. Sterols were determined by precipitation with digitonin, and by the Liebermann-Burchard reaction on the saponified extracts. In the period prior to the infusion of fat, the daily excretion of sterols giving the Liebermann-Burchard color reaction, and expressed as cholesterol, was 0.16 gram, and the total sterol precipitated by digitonin was 0.30 gram. The fecal excretion of sterols during the 10th to 13th day of fat infusion, and between the 21st and 25th days of infusion, averaged 0.42 gram cholesterol and 1.48 grams total sterols daily. Thus the dog was excreting approximately all of the infused sterol (about 1.00 gram cholesterol daily) during this stage of the infusion experiment. The cholesterol to coprosterol ratio is roughly in accordance with that found by Schoenheimer (17). It seems clear, therefore, that the earlier appearance of liver impairment in the dogs of Experiments 1 and 2, in which the emlusion containing cholesterol was used (Emulsion 11MC), should not have occurred primarily from inability to excrete the sterol. In addition, there was no apparent difference in the histopathologic appearance of the tissues of the dogs of Experiments 2 and 3. Thus it would seem that no primary toxic effect can be attributed to the cholesterol.

These observations suggest that the earlier appearance of liver dysfunction in Experiments 1 and 2, when Emulsion 11MC containing cholesterol was used, occurs only when failure of liver function accompanying prolonged fat infusions is present. Since the excretion of sterols into the intestinal tract is initiated in the liver, any slight failure of liver function should result in reduced capacity to excrete the infused sterol, and it would be expected to accumulate in the liver and blood. The sterolemia in the dogs of Experiments 1 and 2 has already been mentioned. The cholesterol content of the liver of the dog in Experiment 2 was found to be 23.0 mgm. per gram of dry liver, as compared with the normal range of 9.0 to 16.1 mgm. in the other dogs of the series of fat infusion experiments reported previously (1).

Inasmuch as the impaired liver function observed after prolonged infusion of the coconut oil emulsions is reversible, as shown in Experiment 1,

it might be expected that no impairment of liver function would occur when the emulsions are used over shorter infusion periods, or at smaller daily infusions. In fact, such was found to be the case in our previous studies (1). Since Emulsion 11MC appears to be more stable, the inclusion of the cholesterol would be desirable, provided the emulsion is not used in quantities which would permit the development of any ill effects resulting from cholesterol. Perhaps smaller quantities of cholesterol would produce the desired stabilizing effects on the emulsion without affecting liver dysfunction.

SUMMARY

1. The daily intravenous administration of coconut oil emulsions to dogs receiving an oral ration adequate in all nutritional factors, but inadequate in calories, prevented further weight loss and produced an increase in nitrogen retention.

2. The infused fat is not lost in urine or feces, nor is it deposited as such in the tissues to any marked extent, except in the cells of the reticuloendothelial system.

3. In 2 dogs in which total carcass analysis of fat was performed, it was found that the body lipids were only slightly displaced in their characteristics toward those of coconut oil. A minimum of 0.72 and 1.56 kgm. of coconut oil could not be accounted for in the tissues of these dogs, and hence must have been metabolized during the experimental period. Thus fat given intravenously to the dog is utilized for energy.

4. Infusion of coconut oil emulsions containing 2.8 per cent cholesterol in the emulsified oil (0.42 per cent cholesterol in the complete emulsion) resulted in impairment of liver function, as measured by bromsulfalein elimination, and a cholesterolemia and increased cholesterol content of the liver when infused at amounts sufficient to furnish 20 to 28 calories per kgm. daily for periods of 4 to 6 weeks.

5. These effects appear to be reversible, since liver function and the cholesterol content of the blood returned to normal when fat infusions were stopped.

6. The histopathologic findings previously described following prolonged infusion of fat were also observed.

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AN HISTOLOGIC STUDY OF THE EFFECTS OF EXPERIMENTAL BOTULINUS POISONING ON THE LIVERS OF GUINEA PIGS

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I. INTRODUCTION

Studies of the effect of Botulinus toxin upon tissue cells have been confined entirely to nervous tissue. Such studies have seemed quite pertinent when it is considered that the main clinical manifestation of Botulinus poisoning is a paralysis of striated muscle. Histologic studies have been made of the entire central and peripheral nervous system with the view of determining the causative factor of this paralysis.

To explain the action of Botulinus toxin in producing motor paralysis, 3 theories have been advanced.

One view has explained the paralysis as due to a thrombosis in the blood vessels of the brain. Such pathology has been reported by Wilber and Ophulus (1) and Dickson (2). However, lesions within the brain, effected by the thrombosis, are not evident. This explanation of Botulinus toxin action has received little support.

In some cases of Botulinus poisoning a degeneration of the nerve cells in the motor nuclei of the spinal cord has been observed (3 to 7). The occurrence of such lesions has led to the belief that the Botulinus toxin directly affects motor nerve cells, and consequently their function is impaired with resulting paralysis. Considerable evidence has been accumulated which fails to support the above theory. First, Cowdry and Nicholson (8) made a very detailed study of the cytology of sensory and motor nerve cells, nerve fibers, and the neuroglia, but were unable, in any of the tissue, to determine microscopic evidence of the action of the toxin. The criterion used for determination of the toxic action of Botulinus was the Nissl substance within the cytoplasm of the nerve cell body. This chromophile substance is extremely labile, and its structure, distribution, and amount are of diagnostic value in determining the condition of the nerve cell. The effect of the toxin is mediated outside of the central nervous system, namely at the peripheral nerve endings, or else the activity is entirely a physiological one, which leaves no cellular manifestations.

Secondly, Dickson and Shevky (5) have reported that, frequently, lesions occur in motor nuclei which are not accompanied by paralysis in the respective muscles innervated by the affected nuclei. Also, lesions may persist in the motor nuclei for at least 2 weeks after the animal has recovered from all clinical evidence of the intoxication.

It thus becomes fairly evident that the motor paralysis resulting from Botulinus toxin is not mediated through the central nervous system. This, then, leaves the only possibility, the action is mediated through peripheral nerves: or at their terminations. Since no lesions along the nerve can be demonstrated, it would appear likely that the nerve endings are affected. Such action has been studied physiologically, as follows:

Edmunds and Long (9) have pointed out that the paralysis effected by Botulinus toxin is very similar to the action of curare, which blocks the nerve impulse at the myoneural junction. Further, when an animal intoxicated by Botulinus toxin is injected with epinephrin or physostigmin, the involuntary muscles of the esophagus, stomach, and intestine act in a normal manner. Epinephrin and physostigmin act on the motor nerve endings as does curare. Such evidence indicates that certain symptoms of Botulinus toxin poisoning, such as constipation, difficulty in swallowing, etc., are not due to an action of the toxin on structures (namely, nerve terminals) located in the gastrointestinal wall. The exact action of the toxin resulting in constipation, etc., has not been accurately determined. The curare-like action of the toxin, together with an absence of microscopal derangement of nerve cells or fibers (8), strongly supports the contention that the activity of Botulinus toxin in some way affects the motor nerve endings. Such structures are exceedingly difficult to study histologically. The only satisfactory

technique for their demonstration is either by impregnations with osmic acid or silver salts, or by methylene blue staining. All techniques are worthless for studying structural differentiations, for the entire organ is uniformly colored. Hence, it is very doubtful if any changes within the nerve endings, as caused by Botulinus toxin, can be demonstrated. The change, or action, is most probably a physiological one with little cytoplasmic change, as suggested by the action of many drugs upon the nervous system.

A review of the literature relative to the effects of Botulinus toxin upon tissues has shown a complete absence of experimentation on the effect of toxin on non-nervous tissue. In the early stages of this work it was noted that in toxin-injected animals, such organs as the liver, kidney, spleen, and intestinal tract showed marked abnormalities. The liver has been selected for this report because the tissue changes are more striking than any of those occurring in the above-mentioned organs.

II. MATERIALS AND METHODS

Normal, healthy guinea pigs were used for this experiment. Their average weight was from 280 to 325 grams. Both male and female animals were used.

Injections were made with *Cl. botulinum* toxin, a thermolabile extracellular toxin-strain A. The toxin was obtained by raising the organisms in veal-infusion broth. The cultures were passed through a series of 3 daily transfers in the media. They were then grown for 14 days in approximately 900 ml. of the same media in a 1-liter flask. To avoid an increase in acidity during sterilization, the broth was prepared without dextrose, and just before inoculation sterile 20 per cent dextrose solution was added to give a 2 per cent concentration. All cultures were incubated at 35° C. Before filtration, 5 per cent phenol solution was added to the pooled toxin to give a 0.5 per cent concentration. After filtration, the toxin was stored in the ice-box until ready for use. Stock cultures were maintained in dextrose semi-solid beef-infusion agar, pH 7.0.

After securing the toxin, it was standardized by establishing the minimum lethal dose for guinea pigs.

The methods of standardizing and preparing the toxin were similar to that used for diphtheria toxin as stated by Wadsworth (10). The M.L.D. determined for the guinea pig was .0003 ml. Table I indicates the sex, dilution used, time of injection and time of death of each guinea pig used in the determination. The guinea pigs were injected with 2 ml. of the toxin dilution.

After the animals were injected, they were held under very close observation, and notations of symptoms were made.

TABLE I

Results of injections of Bacillus Botulinus toxin into guinea pigs to establish the minimum lethal dose (M.L.D.)

No.	Sex	Dilution	Time of Injection	Time of death
1	♀	0.00003	9:15 A.M.	*
2	♀	0.00005	9:30 A.M.	*
3	♂	0.0001	9:45 A.M.	+112 hours
4	♀	0.0002	10:00 A.M.	+108 hours
5	♀	0.0003	10:15 A.M.	−92 hours
6	♂	0.0004	10:30 A.M.	−72 hours
7	♂	0.0005	10:45 A.M.	−47 hours

* animals lived.
− death occurred before 96 hours.
+ death occurred between 96 hours and 5 days.

The first symptom to appear was a difficulty of the animal in swallowing; this was probably due at least in part to the paralysis of the pharyngeal muscles. Simultaneously with an increase in the difficulty of breathing, mydriasis was noted. This indicates a loss of accommodation for near vision, and imperfect function of the oculomotor nerve. Progressive muscular weakness was very marked, resulting in the inability of the animals to lift their limbs, to move about in their cages, or, in later stages, to lift their heads. The weakness in breathing was probably due to a paralysis of the diaphragm. Respiratory movements tend to be lost first from the lower regions of the thorax. In later stages, the movement of the upper ribs becomes gradually diminished until respiration stops. All the animals, regardless of sex, displayed identical symptoms.

All animals, regardless of dilution used, showed the same period of action of toxin prior to death. Thus, an animal injected with a high dilution of toxin would live longer than an animal injected with a low dilution. But prior to death, both animals would exhibit symptoms over the same period of time.

The animals at the point of death were immediately killed and dissected. The tissues removed include the vagus and recurrent nerves, liver, large and small intestines, kidney and spleen. After removal of the tissues from the animal, the pieces were placed in 2 separate fixatives, Bouin's and Zenker's. These 2 fixatives were used in order to observe the difference, if any, in the tissue due to fixation. After fixation the tissue was dehydrated and embedded in paraffin. All sections were cut at 8 micron. The sections were mounted and stained in either Heidenhain Azain or Harris's hematoxylin.

III. OBSERVATIONS AND DISCUSSIONS

A. The histology of the normal guinea pig liver

Blood circulation through the liver is of distinctive distribution. At the periphery of the lobules, embedded in considerable connective tissue, are the interlobular veins (Figure 1). In the
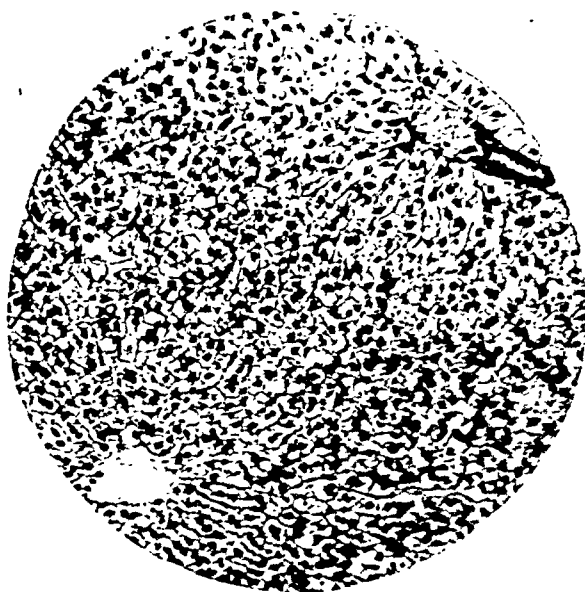
PLATE I

FIG. 1. A SECTION OF LIVER TISSUE OF A NON-INJECTED GUINEA PIG.

This shows arrangement of the tissues in the lobule, central vein, and interlobular vein.
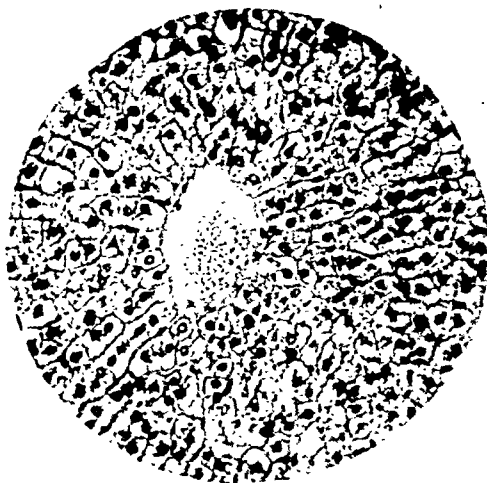
FIG. 2. CENTRAL VEIN OF PREVIOUS FIGURE ENLARGED TO SHOW ARRANGEMENT OF CELL CORDS.
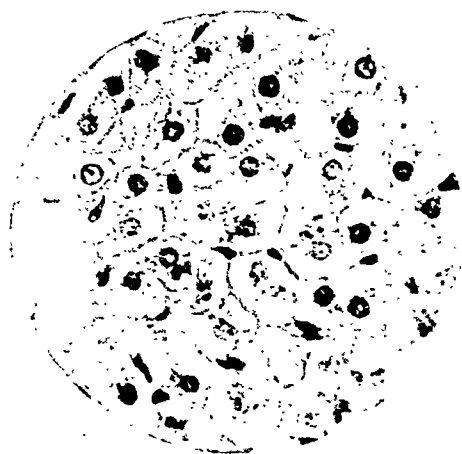
FIG. 3. CELLS IN THE REGION OF THE CENTRAL VEIN.

This shows finely dispersed chromatin in the nuclei, homogeneous cytoplasm with reticular framework, and sinusoids with endothelium.
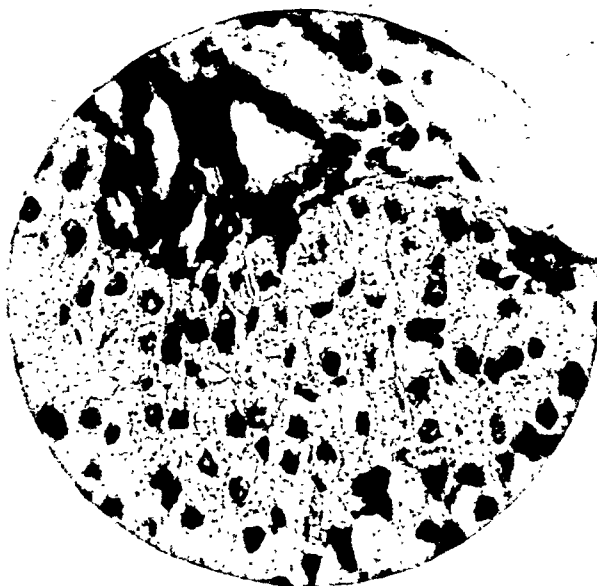
FIG. 4. A SECTION IN THE REGION OF THE PORTAL CANAL.

This shows interlobular bile ducts surrounded by cuboidal epithelium of an injected animal.

center of the lobule occurs a single relatively large vein, termed the central vein, a branch of the hepatic vein (Figures 1 to 3, 6 to 8). These veins may be distinguished from the interlobular veins by their size and slight amount of surrounding connective tissue.

The hepatic cells radiate in cords or traneculae, from the central vein to the periphery of the lobule. The periphery may be marked by a slight accumulation of connective tissue and the presence of the afore-mentioned interlobular veins. The arrangement of cells in cords, however, is not as evident in the periphery of the lobule as in the region of the central vein. Between these cords are blood spaces, the hepatic sinusoids, lined with endothelium. These sinusoids are minute channels, frequently distinguished only by the occasional endothelial cell, or blood cells, along their course (Figures 2, 3).

The hepatic cords are made up of cells which are polyhedral in shape, having 6 or more surfaces. The cells contain a single large spherical nucleus. Occasionally binucleation occurs.

The chromatin material in the nucleus is finely dispersed, with few chromatin knots, or masses. Usually each nucleus contains a single, or possibly 2 or 3, chromatin nucleoli (Figure 3). The cytoplasm of the hepatic cells appears homogeneous after the routine fixatives and stains. Occasionally small vacuoles (remnants of either fat or glycogen) are visible. The finely granular cytoplasm shows a very definite reticular framework (Figure 3).

One of the most important functions of the hepatic cell is to secrete bile, which aids in the hydrolysis of fat. This secretion is conducted by a delicate arrangement of fine bile capillaries, which may be demonstrated with suitable techniques. These capillaries parallel the sinusoids, and their walls are formed by the adjoining liver cells. The bile canaliculi, as they are called, receive short lateral branches which extend between the adjoining liver cells. The fine bile capillaries communicate with the interlobular bile ducts located at the periphery of the lobule and usually adjoining the interlobular veins (Figure 4). The lumena of these ducts are surrounded with cuboidal epithelium. The bile ducts fuse to form the common bile duct. This is joined by the cystic duct from the gall bladder to form the common bile duct. This unites with the pancreatic duct and passes into the duodenum at the ampulla of Vater.

B. The histological effect of B. toxin upon the hepatic cells

The histological appearance of the liver, after toxin injection, shows a very marked effect by the toxin, more so than any other organ studied.

When the body cavity was opened the liver was examined to determine the presence of external lesions. However, the only change noted was a darkening of the liver tissue, probably due to the diffusion of large amounts of blood.

In histological preparations it was noted that there were large areas in which complete necrosis had set in (Figure 5). These areas showed a general breakdown of hepatic cells. The spaces formed by this breakdown were filled with necrotic tissue, degenerate nuclei, connective tissue fibers, blood plasma and blood cells.

After a closer microscopic examination of individual lobules, it was noted that degeneration of cells began in the regions of the central veins (Figure 6). The cells in the region of the interlobular veins showed little effect of the toxin (Figure 7). The extent to which necrosis had progressed in the lobules was variable, some regions being of more advanced stages than others (Figures 4, 5, 7). In those affected to a lesser degree by the toxin, the only noticeable change was a very slight vacuolization of the cells and little or no effect on the surrounding blood vessels, etc. Other cells, effected to a greater extent by the toxin, exhibited conspicuous vacuolation of the cytoplasm, and the concentration of the nuclear material. This is clearly demonstrated in Figure 6.

Figures 6 and 8 show various stages of necrosis in the region of the central vein (Figure 8 to a greater extent than Figure 6). These may be compared to Figure 7, which exhibits a region around the vessels in the periphery of the same lobule from which Figure 8 was made. This, again, shows the greater amount of necrotic tissue around the central vein of the more advanced stages of degeneration. The bile ducts show little or no effects of the toxin.

The characteristic effects of the toxin is best illustrated in Figure 8. This shows a complete breakdown of the central vein, sinusoids and bile

FIG. 5. A BREAKDOWN OF HEPATIC TISSUE AS THE RESULT OF INJECTION OF BOTULINUS TOXIN.

The resulting spaces are filled with necrotic tissue, degenerating nuclei, connective tissue and blood cells.
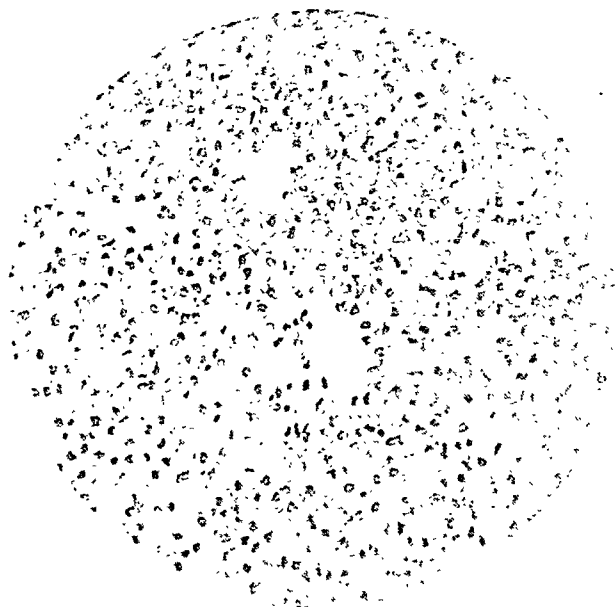
FIG. 6. CELLULAR DEGENERATION OF THE REGION SURROUNDING THE CENTRAL VEIN AS THE RESULT OF INJECTING BOTULINUS TOXIN.
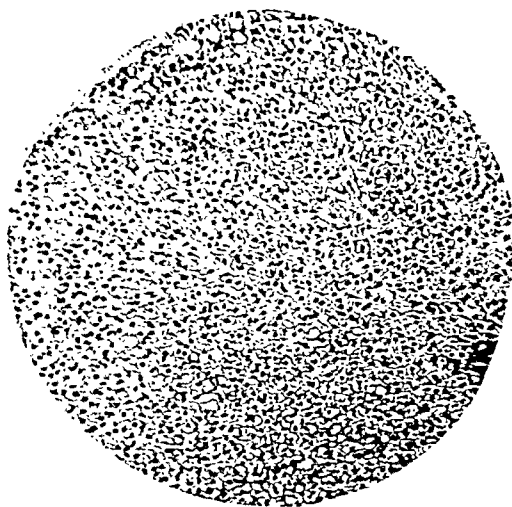
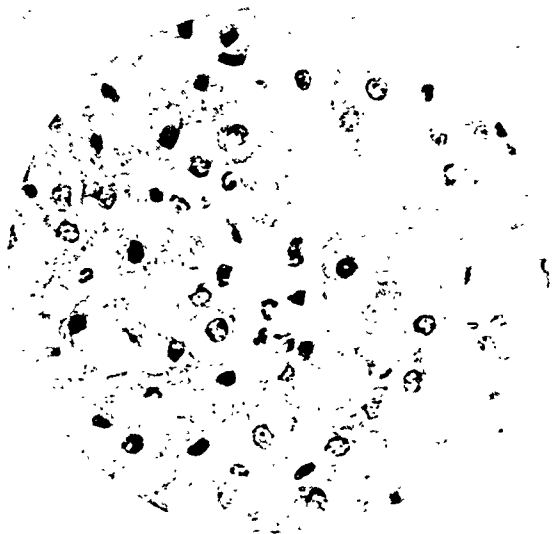FIG. 7. GREATEST CELLULAR DEGENERATION IN THE ENTIRE LOBULE MAY BE SEEN IN THE REGION OF THE CENTRAL VEIN.

FIG. 8. ADVANCED STAGES OF DEGENERATION IN THE REGION OF THE CENTRAL VEIN.

This shows sinusoids, bile capillaries, and supporting connective tissue destruction resulting in infiltration of blood through the areas. Cytoplasmic vacuolization, accompanied by nuclear fragmentation and condensation of chromatin material (pyknosis), is in evidence. Polymorphonuclear leukocytes and erythrocytes are more stable, showing little, if any, effect of the toxin.

capillaries, and complete destruction of the endothelial cells lining these vessels and spaces. A breakdown of these vessels results in a great infiltration of blood throughout this area of necrotic tissue. The supporting connective tissue in this region does not have its characteristic arrangement, but is distributed in the form of irregular bundles.

Areas which show variable effects of the toxic action offer a means of studying the progress of degeneration in the hepatic cell. A study of such regions clearly demonstrates that cytoplasmic vacuolization represents the method of degeneration of the cell. This cytoplasmic vacuolization is accompanied by marked changes in the nucleus, which result in condensation of chromatin material (pyknosis) and fragmentation of the nucleus (Figure 8). In areas slightly affected by the toxin the cells show an accumulation of minute vacuoles adjacent to the nucleus. Apparently by a fusion and increase of vacuolar material the vacuoles become progressively larger in size to the extent of completely surrounding the nucleus. A few strands of cytoplasm connect the nucleus with the peripheral layer of the cell. This peripheral layer of cytoplasm shows a marked condensation of material as compared to the normal. Its density is greatly increased, as indicated both by a greater affinity for stains and by its optical properties. This condensation may be due simply to the forcing of all cytoplasm to the periphery of the cell by the enlargement of the centrally located vacuoles. As yet, no determinations have been made as to the constituents of the vacuoles in the hepatic cells.

Lipoidal material in the liver cells appears in the form of vacuoles. However, fat vacuoles in cells exclusive of adipose tissue invariably show no confluence of vacuoles. Since, in the liver cells affected by Botulinus toxin, the final result is an individual large vacuole formed by the fusions of smaller ones, it is doubtful if the vacuolar material could be fat.

That the vacuolar material may be an accumulation of toxin is suggested by the work of Schneider and Fisk (11). These workers developed a method of testing for the presence of Botulinus toxin in the blood and tissues after death. Such procedure is valuable in diagnosing accurately suspected cases of Botulism. Schneider and Fisk

(11) were able to demonstrate the presence of toxin in the liver in all cases of death due to Botulism. At the same time, toxin could not always be identified in the blood. This evidence strongly suggests that the liver functions to withdraw the toxin from the circulating fluids, and thus the possibility that the toxin is accumulated within the vacuoles of the hepatic cells (Figures 6 and 8).

Some cells have degenerated to the extent that all that remains of the cell is a mass of the nuclear chromatin with no evidence of nuclear wall or cytoplasm. Leukocytes, which tend to be numerous in this region, appear to be more stable in regard to the toxin. Polymorphonuclear leukocytes are the predominant type (Figure 8). The red cells showed no visible effect of the toxin.

SUMMARY

1. An histological examination of the liver of guinea pigs at death from Botulinus toxin has been made.

2. The effect of the toxin is noticed particularly in regions adjacent to the central vein. Occasionally cells adjacent to the interlobular veins are affected.

3. The toxin acts first to produce a vacuolization of the cytoplasm within the hepatic cells.

4. Vacuolization progressively increases until only the nucleus remains, and an actual rupture or disintegration of the cell wall results.

5. Vacuolization is accompanied in the nucleus by pyknosis and fragmentation of nuclear material, nuclear characteristics of degeneration.

6. Conspicuous areas of complete necrosis of liver cells are present. These areas are marked by a great infiltration of blood plasma and cells. Such areas invariably are adjacent to the central veins.

7. It is suggested that the liver functions to remove the toxin from the circulating fluids, and the contents of the cytoplasmic vacuoles in the hepatic cells represent an accumulation of this toxin.

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THE INFLUENCE OF AMMONIUM ION ON THE PLASMA ATA-
BRINE LEVEL AND ON THE URINARY EXCRETION OF
ATABRINE

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In men taking atabrine, the concentration of the drug in the leukocytes is about 400 times that in the plasma, and 100 to 200 times that in the red blood cells (1). Consequently, any appreciable change in the leukocyte count would be reflected by a change in the atabrine level of the whole blood, even though the level in the plasma and erythrocytes remained the same. For this reason, it has been considered essential to "relate the chemotherapeutic activity of atabrine to its concentration in the plasma, rather than its concentration in the whole blood" (1).

In order to avoid measuring any of the atabrine present in the leukocytes, it has been customary to draw blood into an anticoagulant, centrifuge it as soon as possible (preferably within 10 minutes) for 15 to 20 minutes, and recentrifuge the supernatant plasma for 40 to 60 minutes to remove all white cell fragments. No specifications appear to have been made concerning the anticoagulant used. Brodie and Udenfriend (1) merely state that the blood is drawn into an adequate amount of oxalate. In studies conducted at Fort Knox by the U. S. Army, potassium oxalate was used as the anticoagulant. In the work of a U. S. Army research unit in the Southwest Pacific, sodium oxalate was routinely used.

When an attempt was made to use the Heller and Paul mixture of potassium and ammonium oxalates, which is supposed to have less effect on the properties of the cellular constituents of blood than other oxalate solutions (2, 3), it was found that extremely high plasma atabrine levels were obtained.

This observation led to the experiments here reported, which have shown that the ammonium ion has a specific effect in rapidly extracting much of the atabrine from the cellular elements of the blood, and from tissues. Such an effect occurs

in vivo as well as *in vitro*. It should be mentioned that, after the completion of the present work, the authors learned through personal communication that the preliminary observations concerning the effect of ammonium ion on plasma levels *in vitro* had been independently made by a group of British investigators.

EXPERIMENTAL RESULTS

(1) Atabrine was determined by the single extraction method of Brodie and Udenfriend (1). Urinary ammonia was measured by Folin's aeration method (2).

(2) The preliminary observations need not be given in detail. When blood was prevented from clotting, in exactly the manner recommended for the Phillips-Van Slyke specific gravity determination (3), and the plasma was prepared in the usual way for atabrine assay, plasma atabrine levels of 100 μg. or more per liter, instead of the expected 20 to 30 μg., were repeatedly obtained with plasma from individuals taking the suppressive dose of 0.1 gram atabrine daily.

The potassium-ammonium oxalate solution did not yield fluorescent materials when extracted by the same procedures used for blood and plasma. The effect on plasma level was produced mainly by the ammonium oxalate (Table I). This salt, at a concentration of 0.15 M, rapidly extracted much of the atabrine from the blood cells, so that the plasma level approached that of the whole

TABLE I

The plasma atabrine levels obtained with 3 different oxalate solutions as anticoagulants

Anticoagulant	Atabrine μg. per liter
Sodium oxalate	39
Potassium oxalate	56
Ammonium oxalate	169

Thirty ml. blood were drawn from person taking 0.1 gram atabrine daily. Ten ml. were run into each of 3 tubes containing 1 ml. of a 2 per cent solution of the indicated oxalate. The plasmas were prepared in the usual manner.

[1] Capt. Sn.C.
[2] Ist Lt. Sn.C.

blood (Table II). It is evident from Table III that the ammonium ion itself is responsible for the extraction of the atabrine from the cells, and that a concentration of 2 millimolar of ammonium oxalate (4 millimolar ammonium ion concentration) gave almost the maximal effect. That the ammonium ion did not cause some naturally occurring fluorescent constituent of the blood to be extracted into the ethylene dichloride, was shown by the low value of 9 μg. per liter obtained for the whole blood, collected in ammonium oxalate, of a person who had stopped taking atabrine 8 weeks previously.

(3) Figure 1 shows the relationship which obtains between the concentration of added ammonium ion and the resulting concentration of atabrine in the plasma. For this experiment blood was drawn, from a person taking 0.1 gram atabrine daily, into a suitable quantity of 0.15 M lithium oxalate solution. A small portion was kept for determination of the whole blood level. The rest was divided into 6 portions of 10 ml. each, to 5 of which were added very small volumes of ammonium oxalate solution to give the final ammonium ion concentrations of 0.3 to 6.0 millimolar. The tubes were all set centrifuging within 7 minutes after the blood was drawn, and the plasmas were prepared in the usual manner. All had the same pH. It is interesting to note that if this curve is extrapolated to a plasma atabrine level of

TABLE II

The extraction of atabrine by ammonium ion from the blood cells into the plasma

Blood number	μg. atabrine per liter for:		
	Whole blood	Plasma	
		Sodium oxalate	Ammonium oxalate
1	285	26	151
2	308	33	168
3	151	(lost)	105
4	280	26	238

Twenty-five ml. blood were drawn from each of 4 individuals taking 0.1 gram atabrine daily. Fifteen ml. of each blood was added to a tube containing 1.5 ml. of 2 per cent sodium oxalate, the remaining 10 ml. to a tube with 1 ml. of 2 per cent ammonium oxalate. A portion of whole blood was withdrawn from each of the sodium oxalate tubes. The remainder, and the material in the ammonium oxalate tubes, was then used for the preparation of plasma in the usual way.

TABLE III

The specificity of the ammonium ion in rapidly extracting much of the atabrine from the blood cells into the plasma

Subject	Composition of anticoagulant	Millimolar concentration of added ammonium ion in final mixture	Atabrine
			μg. per liter of plasma
Re	a) 0.15 M lithium oxalate	0	25
	b) 0.15 M sodium oxalate	0	25
	c) 0.15 M potassium oxalate	0	36
Ba	a) 0.15 M sodium oxalate	0	33
	b) 0.14 M sodium oxalate 0.01 M ammonium oxalate	1.8	127
	c) 0.13 M sodium oxalate 0.02 M ammonium oxalate	3.6	155
Bu	a) 0.15 M sodium oxalate	0	25
	b) 0.10 M sodium oxalate 0.05 M ammonium oxalate	9.1	83
	c) 0.05 M sodium oxalate 0.10 M ammonium oxalate	18.2	120
Tr	a) 0.15 M sodium oxalate	0	20
	b) 0.15 M ammonium oxalate	27.3	125
	c) 0.07 M sodium citrate 0.08 M sodium sulfate	0	16
Br	a) 0.07 M sodium citrate 0.08 M sodium sulfate	0	18
	b) 0.07 M sodium citrate 0.05 M sodium sulfate 0.03 M ammonium sulfate	5.4	72
	c) 0.07 M sodium citrate 0.08 M ammonium sulfate	14.4	86

Thirty ml. of blood were taken from each of 5 persons taking 0.1 gram atabrine daily. Ten ml. of blood were added to 1 ml. of the various anticoagulant solutions. The plasmas were prepared in the usual manner. No blood was allowed to stand more than 10 minutes before centrifugation was begun.

0, and if the point at which it intersects the abscissa is then taken as a plasma ammonium ion concentration of 0, then the level of 23 μg. per liter (obtained with no added ammonium ion) corresponds to an ammonium ion concentration of 0.1 to 0.15 millimolar, which is in the range of ammonium ion concentration normally found in plasma (4).

In another experiment, blood from a person taking 0.1 gram atabrine daily was run into 5 per cent of its volume of 0.15 M lithium oxalate. The blood was centrifuged immediately, the plasma drawn off and the cells resuspended in 0.9 per cent NaCl solution to make the original volume. The cell suspension was divided into 2 parts, to 1 of which was added enough ammonium chloride solu-
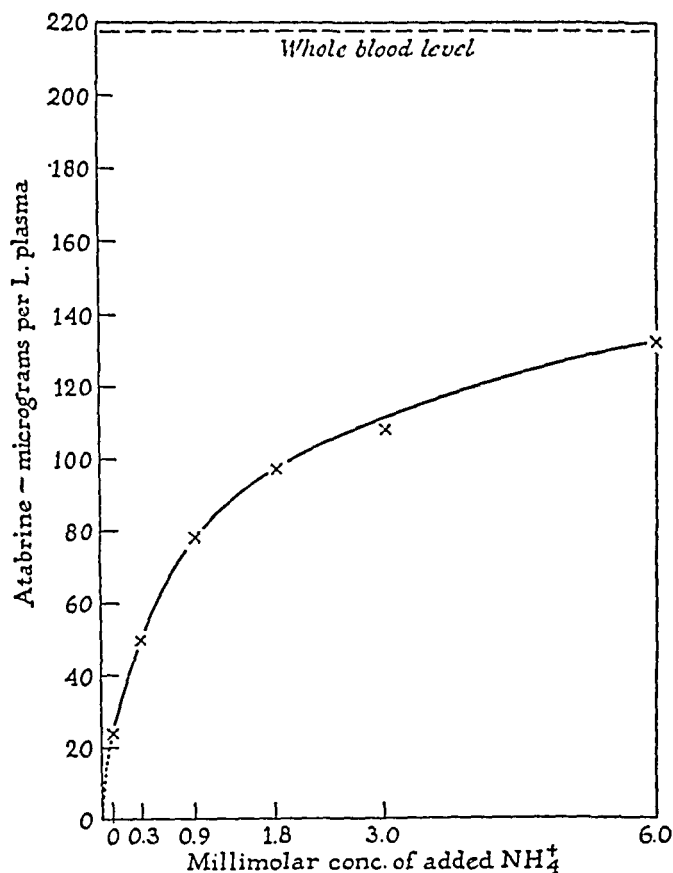
FIG. 1. THE RELATION BETWEEN THE CONCENTRATION OF ADDED AMMONIUM ION AND THE RESULTING CONCENTRATION OF ATABRINE IN THE PLASMA

tion (0.1 ml. to 10 ml. suspension) to give an ammonium ion concentration of 0.1 millimolar. Both suspensions were centrifuged 10 minutes, and the supernatant fluids analyzed for atabrine, with the results shown in Table IV.

TABLE IV

Extraction of atabrine from blood cells resuspended in salt solution

Material	Atabrine μg. per liter
Supernatant from cell suspension in 0.9 per cent NaCl solution	7
Supernatant from cell suspension in 0.9 per cent NaCl solution plus 0.1 millimolar NH^+_4	14

Blood from person taking 0.1 gram atabrine daily.

In 1 experiment, the addition, to a portion of freshly drawn blood, of enough urea to give a 34 millimolar concentration of NH_2, resulted in a plasma level of 31, as compared with a level of 25 for the untreated blood. This was a much smaller increase than that obtained following the addition of considerably lower concentrations of ammonium salts.

(4) In order to note the effect of ammonium ion on the extraction of atabrine from tissues other than blood cells, comparable washed and weighed fragments of liver, removed from rats which had been fed atabrine for 2 weeks, were placed in 0.9 per cent NaCl solution and in 0.9 per cent ammonium chloride solution. The tissue was then removed, and the per cent of atabrine extracted from it was determined by measuring the concentration of atabrine in the fluid which had bathed it, and in the liver tissue. The results are given in Table V. Although the percentage of atabrine

TABLE V

Extraction of atabrine from liver tissue

Exp. no.	Solution	Conditions of extraction	Atabrine extracted
			per cent
1	0.9 per cent NaCl	7 hrs. in refrigerator	0.4
	0.9 per cent NH_4Cl		0.8
2	0.9 per cent NaCl	1 hr. at room temperature	0.5
	0.9 per cent NH_4Cl		1.3

extracted, even in the ammonium chloride solution, appears to be small, it must be realized that the figures are based on the total amount of atabrine in 10 ml. of fluid, as compared to the total amount originally present in a rather thick liver fragment, very rich in atabrine, and with only a relatively small surface actually exposed to the extracting fluid.

(5) In view of the effect of added ammonium ion *in vitro* on the relative distribution of atabrine between cells and plasma, it seemed probable that increasing the ammonium ion concentration of the blood *in vivo* should withdraw atabrine from the tissues into the plasma, and should increase the urinary excretion of atabrine. Such an increase in ammonium ion concentration might also produce a rise in the plasma level of atabrine.

Observations on a number of individuals have shown beyond doubt that there is a close relationship between the amounts of ammonia and atabrine excreted in the urine. An increase or decrease in the ammonia is accompanied by a corresponding increase or decrease in the atabrine. This is true for individual urine samples, as well as for 24-hour excretions. A typical result is

TABLE VI

The effect of sodium bicarbonate and citrate on the urinary excretion of ammonia and atabrine

Subject	24 hour period	Treatment	Excreted	
			Ammo-nia	Ata-brine
			mgm.	*mgm.*
F	1st day	None	706	4.0
	2nd day	None	452	2.9
	3rd day	10 grams sodium bicarbonate plus 10 grams sodium citrate daily.	119	0.7
	4th day	10 grams sodium bicarbonate plus 10 grams sodium citrate daily.	32	0.2
	8th day	5 grams ammonium chloride daily beginning on 7th day.	475	2.2

Twenty-four-hour urinary excretions. Subject F had discontinued atabrine 3 weeks before Period 1, and had been receiving ammonium chloride up to 2 days before Period 1. No atabrine during period of experiment.

shown in Table VI. This subject had been receiving ammonium chloride for about a week up until 2 days before the beginning of the observations. In the first 24-hour period, the ammonia excretion was still high (under the climatic conditions of New Guinea). It fell off during the second day, and the atabrine excretion likewise decreased. Both were then very sharply decreased by the administration of sodium bicarbonate and citrate, in spite of the fact that the volume of urine increased considerably. Several days later, following the administration of ammonium chloride, both the ammonia and atabrine excretions returned to the quantities obtaining on the second day.

Similar results are shown in Figures 2 and 3. The individual (T) who received ammonium chloride showed an increased ammonia and atabrine excretion. It is to be noted that the increase in atabrine excretion continued after the ammonia excretion had begun to decrease. The dosage of sodium acid phosphate taken by individual R was insufficient to increase the ammonia excretion. The atabrine excretion similarly remained unchanged, but on the third day, when the ammonia excretion decreased, the atabrine excretion also decreased. Also shown in Figures 2 and 3 are 4 blood and plasma atabrine levels obtained during the 72-hour period of the observations. In
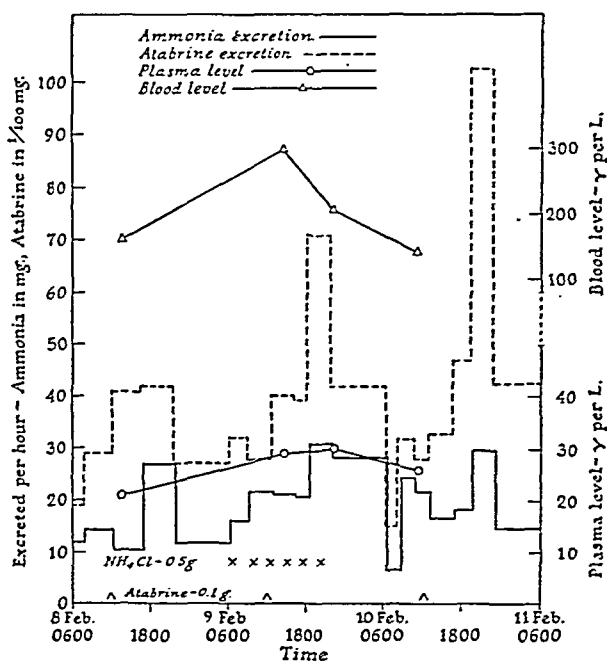
FIG. 2. RATES OF URINARY EXCRETION OF AMMONIA AND ATABRINE, AND BLOOD AND PLASMA ATABRINE LEVELS, OVER A 72-HOUR PERIOD, INCLUDING A PERIOD OF AMMONIUM CHLORIDE ADMINISTRATION

Subject T. 0.1 gram atabrine daily for 8 months.
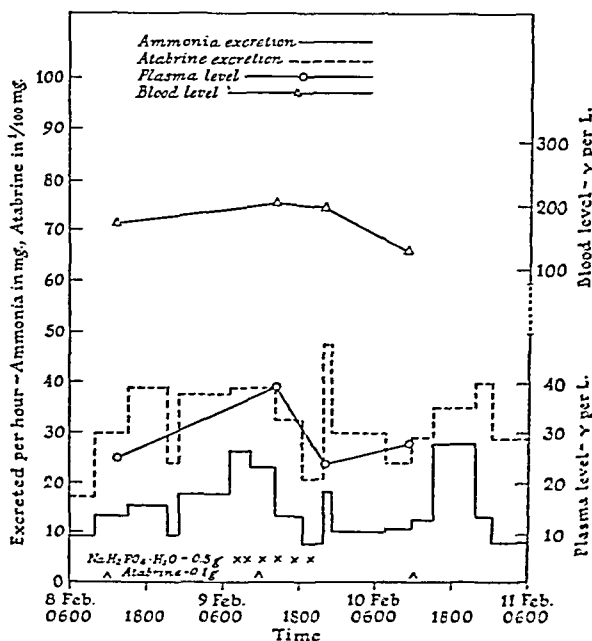
FIG. 3. RATES OF URINARY EXCRETION OF AMMONIA AND ATABRINE, AND BLOOD AND PLASMA ATABRINE LEVELS, OVER A 72-HOUR PERIOD, INCLUDING A PERIOD OF ACID PHOSPHATE ADMINISTRATION

Subject R. 0.1 gram atabrine daily for 7 months.
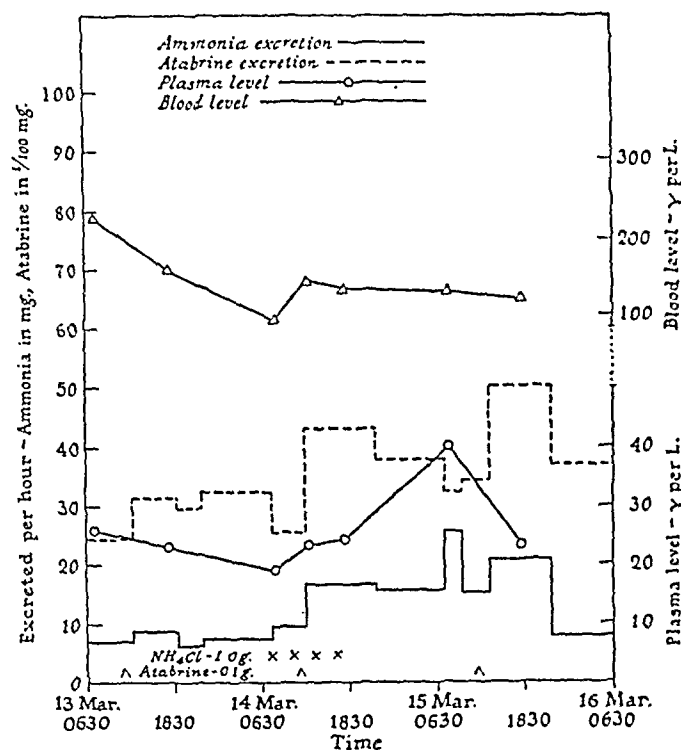
FIG. 4. RATES OF URINARY EXCRETION OF AMMONIA AND ATABRINE, AND BLOOD AND PLASMA ATABRINE LEVELS, OVER 72-HOUR PERIOD, INCLUDING A PERIOD OF AMMONIUM CHLORIDE ADMINISTRATION

Subject T. 0.1 gram atabrine daily for 9 months.
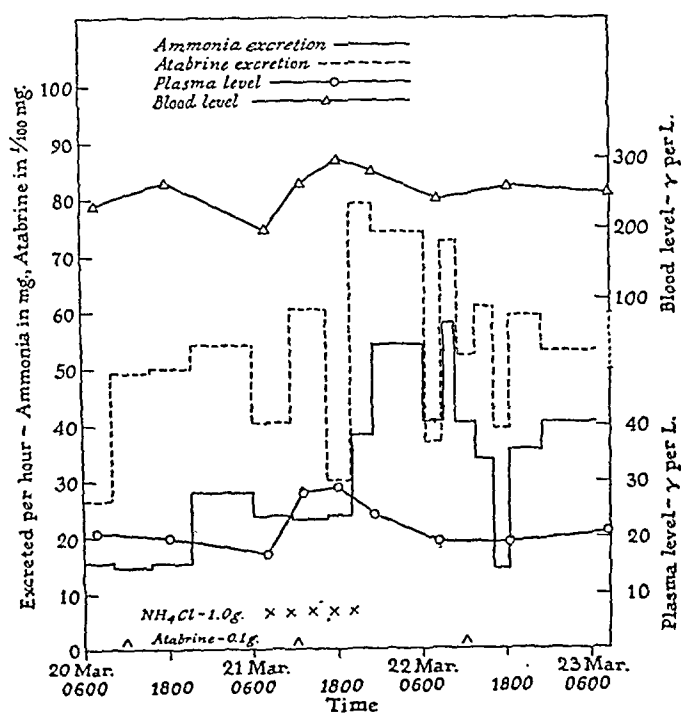
FIG. 5. RATES OF URINARY EXCRETION OF AMMONIA AND ATABRINE, AND BLOOD AND PLASMA ATABRINE LEVELS, OVER A 72-HOUR PERIOD, INCLUDING A PERIOD OF AMMONIUM CHLORIDE ADMINISTRATION

both individuals there was an increase in the plasma atabrine level during the administration of the salts.

The results of further observations of this type are shown in Figures 4 and 5. Subject T again showed a lag between the period of ammonium chloride intake and the period of maximal excretion of ammonia and atabrine. This lag was less marked for subject S. Especially noteworthy are the changes in the plasma atabrine level. In both subjects the level began to rise soon after the beginning of ammonium chloride intake. In subject T the maximum observed level was not reached until early the following day. In subject S the maximum level occurred toward the end of the day of ammonium chloride administration. The changes in the whole blood level were less regular than the changes in the plasma level, but were in the same direction.

DISCUSSION

The effect of ammonium ion on plasma atabrine levels and on the urinary excretion of atabrine is of interest from several standpoints.

(1) The addition of ammonium ion must be scrupulously avoided when blood is being prepared for the determination of plasma atabrine levels.

(2) The amount of atabrine excreted in the urine is closely correlated with the amount of ammonia excreted. Rosenfeld, Jailer and Shannon (5) in 1944 observed that the feeding of ammonium chloride increased the renal excretion of atabrine, while the feeding of sodium bicarbonate decreased it. Emerson and Dole (6) at about the same time noted a correlation between urinary pH and the excretion of atabrine. The former authors attributed the effect to the assumed greater ease of diffusion of the free base, as compared with the acid salts of atabrine, across the cells of the distal segment of the nephron. It seems reasonable to suppose that the specific effect of ammonium ion on the equilibrium between intra- and extra-cellular atabrine also plays a part. A British Malaria Research Unit (7) found that, with a given plasma level, the urinary excretion of atabrine was more closely related to the urinary excretion of ammonia than to the pH or the titrable acidity

which they used to calculate the plasma atabrine level from the determinations of the urinary ammonia and atabrine excretion. The data reported in the present paper, and a considerable volume of additional data which has since been collected in another connection, support the conclusion that urinary atabrine is determined largely by the body level of atabrine, and by the urinary excretion of ammonia. However, the data also show that the relation between urinary atabrine and urinary ammonia is not sufficiently constant in any one individual to permit an accurate estimate of the plasma atabrine level from measurements of the amounts of atabrine and ammonia excreted in the urine.

It would seem likely that increasing the urinary ammonia, as by the administration of ammonium chloride, should increase the rate of elimination of atabrine from individuals who have stopped taking the drug. This may, in certain instances, be of clinical value.

(3) Ever since the study of plasma atabrine levels was begun, it was observed that the plasma level varied among different individuals and in the same individual at different times, even though the dosage was kept uniform (8, 9). The latter variations became less after an individual had been on a particular dosage long enough to reach a balance between the tissue and plasma atabrine concentrations. In the past, it has not been possible by any experimental means (aside from a change in the dosage of atabrine) either to increase or to decrease the plasma atabrine level. The level seemed to vary quite independently of any known factor. The present results indicate that the ammonium ion concentration of plasma might be an important factor in determining the plasma atabrine level. This could be true in spite of the fact that the ingestion of ammonium salts does not materially influence the overall concentration of ammonia in the systemic blood (4). In the first place, as shown in Figure 1, rather small increases in ammonium ion concentration produce marked effects on the atabrine concentration of plasma *in vitro*. In the second place, the ingestion of ammonium salts does increase the ammonia concentration in the venous portal system. Moreover, the increased urinary excretion of ammonia, which follows the ingestion of ammonium salts, is probably accompanied by increased ammonia in the

blood leaving the kidneys, since this blood normally contains about twice as much ammonia as blood entering the kidneys (10). Such local increases in ammonium ion concentration in organs such as the liver and kidney, which have a high concentration of atabrine when this drug is being taken, could conceivably be responsible for an increase in the plasma concentration of atabrine. A complete elucidation of the mechanism whereby the ingestion of ammonium chloride increases the plasma atabrine level of individuals taking atabrine would constitute an extensive physiological study which the authors were unable to undertake.

SUMMARY

1. It has been shown that low concentrations of ammonium ion rapidly extract atabrine *in vitro* from blood cells and from tissues.

2. The urinary excretion of atabrine by individuals taking this drug was found to be closely correlated with the urinary excretion of ammonia. The amount of atabrine excreted in the urine could be increased or decreased by artificially increasing or decreasing the amount of ammonia excreted, as by the feeding of ammonium chloride, or of sodium bicarbonate and citrate.

3. A short period of administration of ammonium chloride to persons on a constant dosage of atabrine resulted in a temporary 50 per cent increase in plasma atabrine level during or just after the period of ammonium chloride intake.

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PLASMA α-AMINO ACID NITROGEN AND SERUM LIPIDS OF SURGICAL PATIENTS

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In the plasma of patients who had acute infections or who had had injuries or operations, the concentration of α-amino acid nitrogen was usually within or below the normal range. The nitrogen metabolism of these patients was studied by Grossman, *et al* (1). Previously Farr and associates (2) had reported that the plasma α-amino acid nitrogen of children decreased immediately after nitrous oxide or ether anesthesia and operation. It seemed likely that such a reduction of amino acids might be a general reaction to injury, and that it might be connected with the nitrogen losses usually encountered in disease and after trauma (1, 3 to 5).

For this reason, the α-amino acid nitrogen of the plasma of 30 patients was measured in the post-absorptive state on the morning of the day of operation, the following morning, and on some subsequent morning in the course of recovery. In 10 other patients plasma α-amino acid nitrogen was measured the morning after operation, and on some subsequnt morning during convalescence.

In addition, the serum lipids of 9 patients were measured on the morning of operation, and on 2 succeeding mornings.

EXPERIMENTAL PROCEDURE

Data included in this paper were collected from patients who were studied to learn the immediate effects of injury upon nitrogen metabolism. Operation was chosen because it permitted observations to be made both before and after injury. The exact plan of the experiments is given below, but the investigation followed work already described in detail (1). On the mornings of the day of operation, the day after operation, and some day 2 to 21 days following operation, blood was drawn from patients in the post-absorptive state, was placed in tubes with dry heparain and analyzed for plasma α-amino acid nitrogen by the method of Hamilton and Van Slyke (6, 7). Certain patients were not followed according to this regimen, but the changes are obvious in the table. Routinely, 0.1 mgm. was subtracted from the plasma amino acid nitrogen as a correction for the urea nitrogen. However, no further correction was made in the few instances in which the nonprotein nitrogen exceeded 40 mgm. per cent. In another article (8) data collected simultaneously are given, but these data do not relate quantitatively to the results included in this report. Blood nonprotein nitrogen, creatine and creatinine of serum were determined on the pre-operative and post-operative mornings. All the urine voided during these 2 days was collected and analyzed for total nitrogen, creatine and creatinine. All food and fluids taken by the patients during this period were measured and recorded. No attempt was made to control the treatment, which was left entirely to the surgeons in charge of the cases. In most instances the patients received only salt solution and small amounts of glucose during the first 24 hours, although certain patients were given transfusions. The nature of the operations with a few relevant remarks about the patients are given below.

A83628 M59. Cholecystectomy for chronic cholecystitis while patient was symptomless.

A44427 M68. Removal of gastric polyp. Operation 20 days after symptoms, brief hemorrhage, an obese patient. Recovery good. On regular diet 36 hours before last blood study.

B44458 F23. Excision of adenofibroma of breast under local anesthesia. Patient in good health.

B61691 M57. Reamputation of leg because of abscess in stump. Infection slight and condition of patient good.

B53965 F67. Cholecystectomy and removal of stone from common duct. Chronically ill and undernourished, slight icterus. Renal insufficiency with elevated nonprotein nitrogen. At last blood study still on soft diet. Not completely recovered.

B8104 M50. Gastrectomy for gastric ulcer. Epigastric distress relieved by meals. No hemorrhage or obstruction. Nutrition well maintained. At last blood study still on full liquid diet.

A44648 F36. Cholecystectomy for cholelithiasis in interval with patient well, quite obese. Febrile at discharge, cause unknown.

B62115 M36. Gastrectomy under spinal anesthesia for gastric ulcer. In good condition. Had severe hemolytic transfusion reaction. Still on full liquid diet at last date.

34340 F52. Repair of abdominal hernia. Condition excellent. Course uneventful.

A11178 F32. Cholecystectomy for cholelithiasis. Condition reported good. Developed infection of stab wound. Only 1 day on soft diet before last blood study.

90248 M69. Left inguinal hernioplasty. Condition excellent. Still on full liquid diet at last blood study.

B69089 F42. Abdominal perineal resection of carcinoma of rectum. Asymptomatic. Condition good. Still on soft diet at last blood study.

25165 F47. Hysterosalpingo-oöphorectomy for uterine myomata.

B68338 F42. Radical mastectomy for carcinoma of breast. General condition good. Stormy post-operative course for 1 week. Final amino acids after 2 days of improvement.

A10607 M34. Inguinal hernioplasty under local anesthesia.

B68027 M34. Inguinal herniorrhaphy. Patient had a carbuncle on his forearm that was incised on the following day.

A79194 F61. Block resection of fibrosarcoma of chest wall, recurrent. Patient had lost weight and had suffered considerably from the tumor. Slow, poor convalescence.

B62484 F37. Cholecystectomy and appendectomy for gall stones and acute appendicitis. Had intermittent fever and chills with almost continuous nausea and frequent vomiting for 10 days before operation. Still febrile and quite sick at time of last amino acid, thereafter satisfactory recovery.

B49302 M43. Excision of pulmonary cyst. Although reported well developed and nourished, he had suffered from severe dyspnea and chest pain for 3 months and had lost 26 pounds. Still quite sick at last blood study.

B59857 M51. Gastrectomy for gastric ulcer. For 2 months he had suffered with epigastric distress, anorexia, nausea, vomiting and tarry stools, and had lost 15 pounds.

61724 M17. Drainage of appendiceal abscess 15 days after onset of acute appendicitis. Still very sick. Eggnog mixture by stomach tube.

9093 M61. Gastrostomy for esophageal carcinoma. Emaciated and chronically ill. Stormy post-operative state at time of last blood study.

B68658 F59. Cholecystectomy for cholelithiasis and chronic cholecystitis. Although obese she had suffered from symptoms continuously for 19 days before operation.

B69576 M70. Exploratory thoracotomy and gastrostomy for carcinoma of esophagus. Had lost 25 pounds.

B5775 M30. Resection of ileum, cecum, and ascending and transverse colon for enteritis. Also had pulmonary tuberculosis. Slow convalescence. Only 2 days on regular diet at last blood study.

B68445 M65. Resection of sigmoid for carcinoma 2 weeks after a cecostomy. Still quite stormy post-operative course at last blood study.

B61943 F46. Drainage of liver abscess which followed severe traumatic injury 3 months earlier.

34038 F35. Appendectomy, 11 hours after onset of acute appendicitis.

A94807 M66. Cholecystojejunostomy for carcinoma of duodenum. Had been jaundiced for 2 months and had lost much weight. Still quite sick at last blood study.

B68886 F52. Drainage of lung abscess, sequel to a pneumonia that began 19 days earlier. Convalescence satisfactory but slow.

A36925 M22. Appendectomy 48 hours after onset of abdominal pain and nausea. Normal appendix removed. Patient well developed and well nourished.

A49273 M48. Right inguinal hernioplasty under local anesthesia in an obese patient who had not been ill.

B56452 M18. Appendectomy 13 hours after onset of acute appendicitis in a well developed and well nourished patient.

B19794 M45. Ventral herniorrhaphy following satisfactory recovery after penicillin and excision of axillary abscess 17 days previously. Moderately obese.

B58111 M20. Appendectomy 24 hours following onset of acute appendicitis in a well developed and well nourished male.

B58721 M37. Herniorrhaphy after 10 year history of right inguinal hernia which had not interfered with strenuous muscular work. Nothing by mouth or parenterally during first post-operative day.

A3107 M53. Left inguinal herniorrhaphy in a well nourished patient with arteriosclerotic heart disease and earlier history of pulmonary fibrosis.

B57895 M52. Appendectomy 48 hours after onset of appendicitis with vomiting and ingestion of fluids only. Patient thin and had chronic bronchitis. Post-operatively wound in-

fection developed and patient febrile for 7 days, after that good recovery.

91253 M25. Appendectomy 11 hours after acute onset of appendicitis. Well developed and very well nourished. Post-operatively febrile for 5 days with drainage from wound site. Six days later wound had healed and patient was discharged

B57836 M32. Stab wound in chest of a well developed, well nourished male. Following supportive treatment for shock, diaphragm and peritoneum were sutured. Bronchopneumonia developed, sulfadiazine given, wound continued to drain. Febrile course for 2 weeks followed by improvement.

RESULTS

Plasma α-amino acid nitrogen was measured by the same procedure (6, 7) 106 times on 55 patients. Among these patients were most of the 45 patients studied by Grossman, Sappington, Burrows, Lavietes and Peters (1). About half of the 45 had medical conditions (meningococcus meningitis, pneumonia, scarlet fever, etc.) while the others had surgical conditions. In Figure 1 the post-absorptive concentrations of α-amino acid nitrogen in the plasma are compared with the nitrogen metabolim. There is obvious lack of correlation. Even when nitrogen catabolism and negative nitrogen balances were extremely large, plasma amino acid nitrogen was most frequently in the normal range or even below normal.

In Table I are given the concentrations of α-amino acid nitrogen in the plasma of surgical patients on the morning of the operation, and of the first post-operative day, and at various intervals thereafter. In the first column after the patients' numbers the physical conditions of the patients at
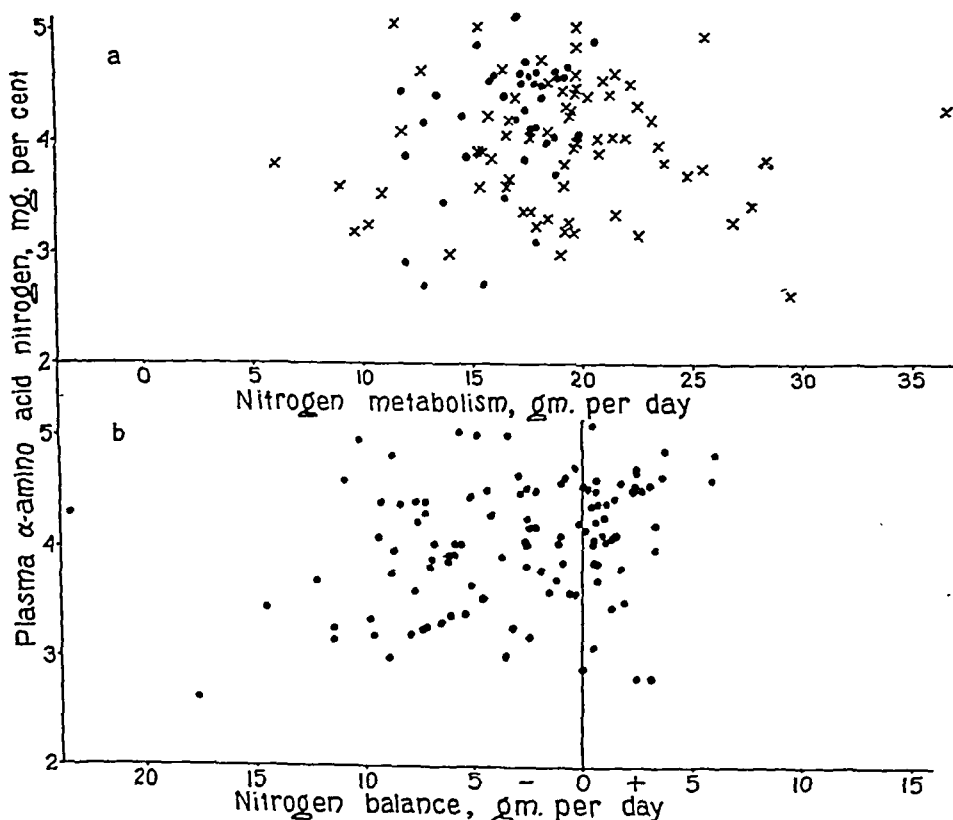
FIG. 1. (a) THE POST-ABSORPTIVE CONCENTRATION OF α-AMINO ACIDS OF PLASMA COMPARED WITH NITROGEN METABOLISM *

(b) THE POST-ABSORPTIVE CONCENTRATION OF α-AMINO ACIDS OF PLASMA COMPARED WITH NITROGEN BALANCE

* Nitrogen intake or output, whichever was the larger. Circles = positive, crosses = negative, balances.

TABLE I

The effect of operation on the concentration of α-amino acid nitrogen of plasma

Number	Physical condition before operation	Plasma α-amino acid nitrogen						Recovery
		Postoperative day						
		0	1					
		mgm. per cent	mgm. per cent			mgm. per cent		
A83628	+	4.95	3.45	4.17 (6)*				+ −
A44427	+	4.85	3.54	4.17 (6)				− +
B44458	+	4.54	4.48	4.36 (2)				+ +
B61691	+	4.53	4.13	4.32 (13)				+ +
B53965	−	4.44	3.97	3.37 (4)	3.81 (6)			+
B8104	+	4.44	3.24	4.81 (7)				+ −
A44648	+	4.39	3.81	3.98 (4)	4.22 (6)	3.49 (15)		−
B62115	+	4.34	4.09	2.93 (2)	2.92 (3)	2.96 (4)	3.63 (10)	+ −
34340	+	4.30	2.71	4.08 (7)				+ +
A11178	+	4.24	3.20	3.75 (7)				+ −
90248	+	4.24	3.74	4.57 (2)				+ −
B69089	+	4.17	3.14	3.99 (9)				+ −
25165	+	4.16	3.64	4.22 (8)				+
B68338	+	4.16	3.00	2.58 (2)	3.99 (4)	4.41 (8)		+
A10607	+	4.14	3.57	3.93 (3)				+ +
B68027	+	3.97	3.73	4.12 (2)				+ +
A79194	−	3.96	3.42	3.51 (7)				−
B62484	−	3.94	3.31	3.87 (2)				−
B49302	−	3.90	3.27	3.33 (2)	4.12 (8)	3.92 (11)		−
B59857	−	3.85	3.03	3.16 (2)	4.24 (9)			+
61724	−	3.84	2.94	4.61†(5)				−
9093	−	3.73	3.41					− −
B68658	−	3.72	3.54	3.74 (8)				+ +
B69576	−	3.70	3.38					− −
B5775	−	3.60	2.93	3.31 (11)				+ −
B68445	−	3.51	2.73	4.22 (4)				−
34038	+	3.27	3.56	4.41 (2)	4.76 (6)			+ +
B61943	−	3.19	2.85	3.74 (9)				−
A94807	−	2.75	3.04	2.93 (5)	2.98 (9)	3.57 (13)		−
B68886	−	2.51	2.71	2.49 (2)	2.88 (6)	3.95 (8)		+
A36925	+		2.90	4.57 (9)				+ +
A49273	+		4.46	4.56 (14)				+ +
B56452	+		3.19	4.54 (8)				+ +
B19794	+	4.39‡	3.18	4.44 (14)				+ +
B58111	+		4.22	4.29 (7)				+ +
B58721	+		2.97	4.26 (12)				+
A3107	+		3.59	4.11 (6)				+
B57895	−		3.14	3.98 (18)				+
91253	+		3.30	3.71 (12)				+ +
B57836	+		2.62	3.59 (21)				+ −
Patients who had pneumoencephalograms and took only small amounts of fluid during the subsequent 24 hours								
PC3452		3.85	3.58	3.68 (2)	3.68 (3)			
PC3474		4.75	4.26	4.41 (2)	4.81 (3)			
PC3477		4.49	4.47	4.38 (2)				
PC3482		3.69	4.02	4.19 (2)	3.60 (3)			

* Figures in parentheses represent number of days after operation.
† Eggnog mixture given continuously by tube at this time.
‡ 17 days previously.

the time of operation are represented, although these are given in more detail in the protocols. A good physical condition is designated +, and a debilitated physical condition is designated −. The first 30 subjects are arranged in the descending order of the initial plasma α-amino acids. It is at once apparent that in general the patients with amino acid nitrogen initially above 4.0 mgm. per cent were in good physical condition before operation, while those with amino acid nitrogen below 4.0 mgm. per cent (with 1 exception, 34038) had been suffering from serious disability or injury for some time before the operative day. The 10 patients who did not have blood studies on the morning preceding the operation are arranged in the descending order of the plasma α-amino acid

nitrogen determined during convalescence, 6 to 21 days post-operatively.

Plasma amino acid nitrogen fell during the 24 hours following operation in all but 4 of the first 30 patients. This fall is in marked contrast to the relative stability of the post-absorptive plasma amino acids of 3 normal subjects. In the course of 1 month the plasma α-amino acid nitrogens of C. W. were 3.73, 3.38, 3.56, and 3.77; of R. C., 4.13, 4.21, 4.30, and 4.23; and of E. M., 4.07, 3.88, and 4.17 mgm. per cent. The average value for all 30 patients on the first post-operative day was 3.39 mgm. per cent of plasma α-amino acid nitrogen. Below this level fell 3 of the 10 plasma α-amino acid nitrogens of the 10 patients whose pre-operative levels were not estimated.

In the first 30 patients the extent of the fall was roughly proportional to the gravity of the operative procedure, except that when the amino acid nitrogen was extremely low at the onset it fell less than when it was initially high. The drop in amino acids was not large in the plasma of 6 patients who had minor operations (A10607, 90248, B68027, B44458, B61691, and A49273) and in 1

appendectomy patient, B58111. In fact, the changes in these patients are comparable with the variations in 4 patients who had pneumoencephalograms and were able to take only small amounts of fluid containing about 400 calories during the 24 hours thereafter. These values are given at the end of Table I.

In all 40 patients during recovery amino acid nitrogen rose, approaching or exceeding its pre-operative concentration in the 30 patients in which this was ascertained.

The serum lipids, measured by methods previously described (9), of only 8 patients are shown in Figure 2. The ninth patient who died shortly after operation, had pronounced disease of the liver and is, therefore, omitted. In every instance the serum lipids fell after operation. In this drop, lipid phosphorus and both fractions of cholesterol were involved in every instance; the fatty acids of neutral fat, in 6 of 7 cases. The decrease was usually greater on the second than on the first day. The decrements were small, but unequivocal and consistent. In no instance did the concentration of any lipid constituent fall below the normal
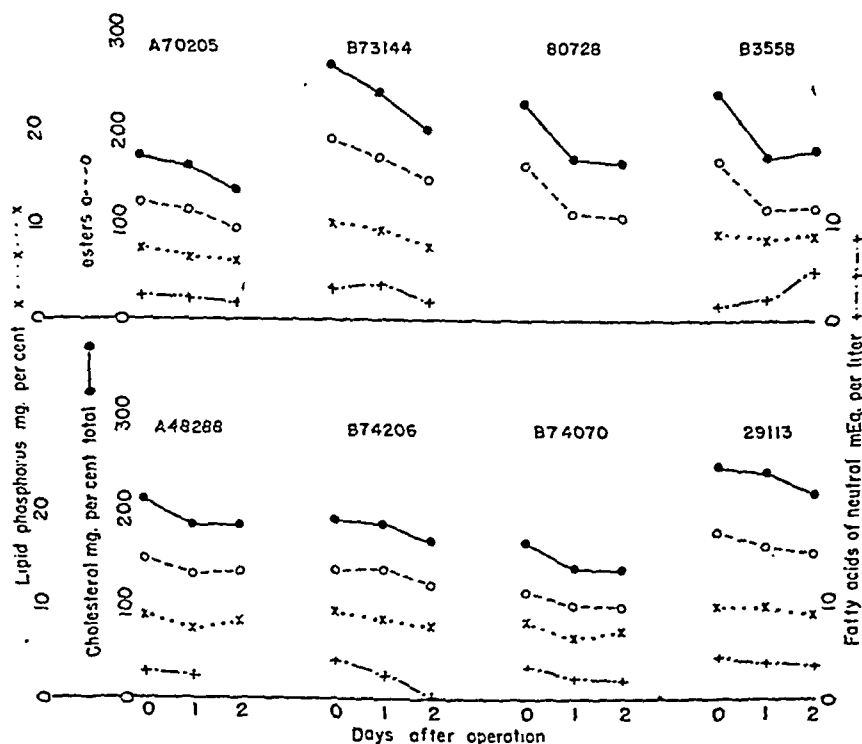
FIG. 2. SERUM LIPIDS ON PATIENTS THE MORNING OF OPERATION AND ON 2 SUCCEEDING MORNINGS

range of variation. Because all lipid fractions were affected, their relations to one another were not significantly disturbed. The ratio, free cholesterol: total cholesterol, on 2 occasions rose to 0.34, just above the normal range.

DISCUSSION

The relation of the behavior of the amino acids to the conditions of the patients is best illustrated by comparisons of similiar cases. There were 7 operations on the gall bladder and bile ducts which can be compared. The initial α-amino acid nitrogens of B62484, B68658 and A94807, who had all been seriously ill prior to operation, were 3.94, 3.72, and 2.75 mgm. per cent, all below the average normal value of 4.23 mgm. per cent (7). On the other hand, A83628, A44648, and A11178, who were in good condition and free from symptoms before operation, had initial amino acid nitrogens above 4.23 mgm. per cent. The amino acids of this latter group fell sharply after operation, while those of the more chronically ill patients were but little affected. B53965 is an exception. Her initial amino acid nitrogen was high, although she was chronically ill, jaundiced and wasted. This patient, however, had definite renal insufficiency with a blood nonprotein nitrogen of 41 mgm. per cent which rose to 50 after operation. It has been reported that plasma amino acid nitrogen may be elevated in patients with advanced renal insufficiency. In addition, the amino acid nitrogen was not corrected for the elevated blood urea. In this connection it may be significant that the amino acid nitrogen of B62115, who had a temporary renal insufficiency as the result of a hemolytic transfusion reaction, did not fall strikingly until the second post-operative day.

The series of gastric operations affords equally good contrasts. B8104 and B62115, who were symptomless and in good condition at the time of operation, had initial plasma amino acids of 4.44 and 4.34 mgm. per cent. B59857, 9093, and B69576 were in deplorable states. Their amino acids were 3.85, 3.73, and 3.70 mgm. per cent. Of the hernia patients, only B68027 had low initial amino acid nitrogen. This patient also had on his forearm a carbuncle that required incision the following day.

The amino acid nitrogen of B44458 did not fall appreciably after excision of an adenofibroma of

the breast under local anesthesia. On the other hand, amino acid nitrogen fell sharply from an initially normal concentration in B68338 after a radical mastectomy. In A79194, who was suffering from a recurrent fibrosarcoma of the chest wall, the amino acid nitrogen was low before operation, and fell further after a block resection. The last 2 patients, A94807 and B68886, were desperately ill before operation. Amino acid nitrogen in both was extremely low. Under these circumstances it did not fall further after operation. The low initial concentration in 34038 is quite inexplicable. The patient had been sick only 11 hours with acute appendicitis. Her post-operative course was uneventful. She differs from all the other cases also in the fact that the amino acid nitrogen of her plasma rose steadily after operation.

After injury, the duration of the amino acid nitrogen depression seems to be proportional to the severity of the operation and the post-operative course. This may be seen in the table, in which the degree of recovery at the time of the final plasma amino acid estimation has been designated in the last column. Special notes about recovery are given in the protocols, and no mention of recovery indicates that the course fell into the categories listed below. Prompt recovery in a vigorous individual was graded ++; satisfactory recovery accompanied by some debility was graded +; convalescence in which the patient was holding his own, but gaining slowly, or was unable to take a full regular diet, was marked ±; unsatisfactory recovery was classed as —. For example, the secondary drop in A44648 was associated with a febrile reaction, the cause of which was not discovered. That the degree of recovery was associated with a rise in plasma amino acid nitrogen is indicated by the average values for the 4 recovery groups of patients. The 14 patients in the ++ group had an average final plasma α-amino acid nitrogen of 4.27 mgm. per cent; the 8 subjects in the + group, an average value of 4.16; the 8 patients in the ± category, an average of 3.85, and the 7 patients in the — group, a value of 3.76 mgm. per cent. Examination of individual cases with a stormy post-operative course reveals the prolongation of low levels of amino acid nitrogen until recovery progressed. Thus, B68338 was extremely ill after a radical mastectomy. Her plasma amino acids dropped from a pre-operative value of 4.16 mgm.

per cent to 3.00 on the first post-operative day, and to 2.58 on the second post-operative day. Two days later the amino acid nitrogen had risen to 3.99; and 4 days thereafter, to 4.41 mgm. per cent. B49302, whose pre-operative amino acid nitrogen was 3.90 mgm. per cent, after pneumonectomy had amino acid nitrogens of 3.27 and 3.33 mgm. per cent on the first and second post-operative days. Six days after this the plasma α-amino acid nitrogen had risen to 4.12 mgm. per cent. B68886 was still acutely ill and febrile 6 days after operation. Her amino acid nitrogen at this time had risen slightly, but only to 2.88 mgm. per cent. A patient, B54752, not in this series, after an appendectomy, developed a fecal fistula and pneumonitis with a question of tuberculosis. For about 6 weeks he was on a daily intake of 80 to 125 grams of protein, and 3000 to 4000 calories. In spite of the fact that he was gravely ill, his nitrogen balance was slightly positive for the last week of this period. Nevertheless, his plasma α-amino acid nitrogen was only 3.39 mgm. per cent at the end of this period. Two days later, when afebrile, the amino acid nitrogen was 3.95 mgm. per cent. That retarded recovery from injury is accompanied by low values of the α-amino acid nitrogen is in accordance with low values observed earlier in this study during the course of infectious diseases, and also reported by Farr and others (10) in adults with pneumococcus pneumonia. However, in infectious diseases our own measurements were not made frequently or systematically enough to establish this thesis. Hypoaminoacidemia during exacerbations of nephrosis in children has already been reported by Farr (11, 12).

Nitrogen balance studies were not carried out during the period of convalescence in all the patients studied. However, in a larger group of patients with infectious diseases or subjected to operation, the post-absorptive concentrations of α-amino acid nitrogen in the plasma have been compared with the nitrogen metabolism and with nitrogen balances (Figure 1). There was no correlation. Even when nitrogen catabolism and negative nitrogen balances were extremely large, plasma amino acid nitrogen was sometimes below normal. An example of a low amino acid after large nitrogen catabolism has been cited (B54752) in the previous paragraph as an example of low

plasma amino acids with lack of recovery. Another example of the lack of correlation between nitrogen metabolism and plasma amino acid level was afforded by a patient with lobar pneumonia (B60476). For 8 days this patient ingested 110 to 173 grams of protein and 2900 to 4200 calories. His nitrogen balance varied from -10 grams to $+3.5$ grams daily. His plasma amino acid nitrogen rose only from 3.75 to 4.19 mgm. per cent.

No comparison was made of the plasma amino acids and the serum proteins or serum albumins in the 30 patients who were studied pre-operatively and during recovery. However, plasma amino acids have been compared with serum albumin in the 10 patients who were studied immediately after operation and late in the recovery period, and on 23 patients with injuries or infectious diseases. No distinct relationship was apparent.

It has been generally presumed that the products of protein metabolism, both in their passage to the liver and tissues for synthesis, and to the liver and kidneys for destruction and excretion, are transported largely in the form of α-amino acids. It might be naturally anticipated, therefore, that the concentration of these compounds would in some measure parallel the rate of nitrogen metabolism. This does not appear to be the case. Although nitrogen metabolism is not greatly increased immediately after operation, it is not strikingly reduced. Moreover the reduction of amino acids that follows operation appears to continue throughout the subsequent period of protein wastage, even when the nitrogen catabolism is extremely large. This suggests may be associated with the phenomenon destruction of protein." In this whole economy of protein is profound Synthetic processes appear to be in tein is routed to destruction. tion of amino acid nitrogen in th dicate that production of urea liver that the tissues are d acids required for the pro

Just as patients with standing injury do not of toxic destruction acids do not fall afte ever, initially depr

It was hoped th
reaction resided in

to this organ might be demonstrated by characteristic disturbances of the serum lipid patterns. The fact that the serum lipids fall after operation adds 1 more item to the growing list of disorders that accompany the injury reaction. This change is not similar to that which occurs in water deprivation and starvation when the lipids increase slightly (13). The nature of the lipid disturbance cannot, however, be used to incriminate the liver, because all the lipid components are proportionally affected, while in liver disease their relations to one another are usually distorted.

CONCLUSIONS

Plasma α-amino acid nitrogen falls abruptly after injury, and remains low until recovery is well advanced, even while nitrogen catabolism is greatly increased by administration of high protein diets. The extent of the abrupt fall is directly proportional to the severity of the operative procedure, and roughly inversely proportional to the initial amino acid concentration. The latter reflects the pre-operative condition of the patient. If this is normal, the plasma α-amino acid nitrogen is greater than 4.0 mgm. per cent. If the patient is seriously ill before operation, the α-amino acid nitrogen is less than 4.0 mgm. per cent before operation. If it is already greatly reduced, it may fall no further as a result of the operation.

After injury serum lipid fractions, total and free cholesterol, lipid phosphorus and fatty acids, diminish by small but unequivocal decrements. acid lipid components are proportionally affected renal in distortion such as occurs in liver disease. transfusion the second p ateful to the Department of Surgery for The series c studies on patients on their service. We ciation to Miss Adelaide Kulinkowski, good contrasts. re, Miss Catherine Marks, and Mrs. symptomless and operation, had init. and 4.34 mgm. per B69576 were in deplo. acids were 3.85, 3.73, a Of the hernia patients, on. tial amino acid nitrogen. on his forearm a carbuncle tl. the following day. The amino acid nitrogen of ᵀ appreciably after excision of

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SOME OBSERVATIONS ON MEN SITTING QUIETLY IN EXTREME COLD

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Military operations in cold weather naturally pose many problems. One of the most important is related to immobilization of men for long periods of time, preventing them from indulging in even the smallest movements from fear of having their presence detected. The experimental work being reported is concerned with the reactions of soldiers sitting quietly for periods of 2 to 3 hours in environmental temperatures ranging from 1° to -40° C.

METHODS

Forty-five young men in excellent physical condition served as subjects for a total of 430 tests. The largest number of exposures for a single individual was 35. Two to 5 tests were performed on each subject in a single environment. One group of 5 subjects was exposed to all environments, with the exception of 1.1° C. The average characteristics of the entire group were as follows: Age, 20.5 years; height, 67.6 inches; weight, 149 pounds; and surface area, 1.79 sq. M.

All exposures were made in the morning between 8 and 12 o'clock. The subjects ate a light breakfast 2 to 3 hours prior to their entrance into the cold room. The men put on thermocouple harnesses of 5 to 15 copper-constantan couples, and after donning their underwear, remained lying quietly for at least ½ hour in a control room, environment 22° C., 50 per cent relative humidity. Basal measurements of skin and rectal temperatures (with a thermocouple or a calibrated rectal thermometer) were then obtained, and the men proceeded to dress in their Artic clothing.[3]

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[2] Tec. 3.

[3] "Artic clothing," with insulative value of approximately 4 clo., consisted of:

Underwear, wool 50-50
Trousers, field, alpaca pile (⅜ inch)
Trousers, field, cotton
Jacket, field, pile (⅜ inch)
Parka, field, pile (⅜ inch)
Parka, field, cotton
Socks, cushion sole, 1 pair
Socks, ski, wool, 2 pair
Shoes, felt or

Muklaks, with burlap insoles
Mittens, wool
Mittens, shell, outer

RESULTS

The data obtained are presented in Tables I to III, and Figures 1 to 5. All data presented in the tables are mean values, but in order to indicate to some extent the variability that was encountered, ranges are also given.

Metabolism of seated, clothed men. When men were quietly seated in a relatively cool environment of 22° C. for a 3-hour period, only slight alterations of approximately 5 per cent in metabolism were observed (Table I). Exposure to cold environments was accompanied by increased metabolic rates, and this was evident even in the very first hour of the sitting period. However, irregularities in response were common, varying from no change to increases of over 30 per cent. This latter value, obtained at an environmental temperature of 1.1° C., was exceptional for the first hour; the majority of the increases were around 10 per cent. During the second hour of exposure a striking rise of the metabolic rate occurred at all environments. The highest increase of 53 per cent was observed at the lowest environmental temperature, − 40° C., although no correlation between ambient temperature and metabolic response was noted. The metabolism continued to rise during the third hour, reaching in the − 40° C. environment a value of almost 74 per cent above basal.

Skin and Rectal Temperature. The data are presented in Tables II and III and Figures 1 to 5. The mean skin temperatures were calculated from the 5 skin areas included in Table II (1). The major portion of fall in mean skin temperature occurred in the first hour of exposure. The average mean curves plotted in Figure 1 illustrate this rapid fall. The rate of decreasing temperatures was very slow in the last half of the exposure. While this may be related to the increased metabolism of the later period, more probably it indi-

TABLE I

Metabolism of men sitting quietly at different environmental temperatures

Room temp. °C	First hour Cal. per M² per hr. Mean	Range	Per cent change*	Second hour Cal. per M² per hr. Mean	Range	Per cent change*	Third hour Cal. per M² per hr. Mean	Range	Per cent change*
22.2	50.5	36 to 68		48.0	33 to 62		49.0	35 to 62	
1.1	66.0	65 to 67	30.7				59.5	56 to 63	21.4
−17.8	53.8	46 to 63	6.5	60.8	50 to 75	26.7	62.8	54 to 80	28.2
−23.3	56.1	43 to 66	11.1	67.8	45 to 99	41.2	79.1	57 to 110	61.4
−26.1	56.0	54 to 58	10.9	66.7	56 to 78	39.0			
−28.9	57.0	38 to 75	12.9	66.0	31 to 99	37.5	68.0	59 to 78	38.8
−34.4	49.8	40 to 59	−1.4	63.0	52 to 84	31.2	84.7	57 to 109	72.8
−40.0	57.3	48 to 74	13.5	73.6	55 to 87	53.3	85.2	66 to 106	73.9

* Per cent change in means, the mean values at 22.2°C being used as references.

TABLE II

Skin temperatures of men sitting quietly in the designated environmental temperatures

	1.1°C Mean	Range	−17.8°C Mean	Range	−23.3°C Mean	Range	−26.1°C Mean	Range	−28.9°C Mean	Range	−34.4°C Mean	Range	−40.0°C Mean	Range
	°C	°C	°C	°C	°C	°C	°C	°C	°C	°C	°C	°C	°C	°C
Mean skin														
Basal	33.0	31.3 34.6	32.8	31.1 34.4	32.7	26.9 35.7	31.5	29.7 33.3	32.5	28.7 34.3	32.8	32.1 33.9	33.0	30.0 35.5
1 hour	32.1	30.7 34.1	30.4	26.7 31.7	28.5	23.4 33.4	29.4	27.9 30.9	27.8	23.7 31.6	28.2	27.2 29.3	26.5	22.4 29.7
2 hours	31.7	28.7 33.0	28.5	25.1 30.6	26.9	22.5 31.5	28.2	27.2 29.2	26.1	22.7 30.3	27.6	25.0 27.6	25.0	20.7 28.6
3 hours			27.2	24.7 29.5	26.0	21.0 30.1			25.5	22.8 29.1	27.0	26.3 27.3	24.5	20.5 27.9
Thigh														
Basal	31.4	26.6 35.2	33.1	30.5 36.0	32.5	21.9 36.8	29.9	20.7 36.4	31.9	25.0 35.9	31.6	28.0 33.8	32.3	28.1 35.7
1 hour	30.5	29.7 31.6	29.9	24.4 37.7	27.8	18.8 35.7	28.0	17.7 34.1	28.4	18.7 35.6	26.9	23.1 30.7	24.9	15.5 29.8
2 hours	29.3	28.6 30.5	28.5	22.0 37.1	26.2	16.3 33.0	26.4	16.4 33.9	27.3	16.9 36.4	25.6	21.7 30.4	23.9	11.9 31.6
3 hours			26.3	21.3 31.7	25.1	17.1 31.5			24.1	18.3 31.6	25.6	21.3 30.1	23.1	12.0 28.6
Toe														
Basal	32.2	30.0 35.9	29.0	15.8 36.0	29.9	16.3 39.9	26.9	16.2 35.1	26.7	9.2 34.8	30.0	22.5 33.6	30.5	21.2 35.4
1 hour	24.3	15.0 30.1	22.9	9.6 30.8	23.2	5.0 34.4	11.9	5.2 20.0	15.8	2.4 30.8	17.0	11.2 25.8	19.4	7.5 26.6
2 hours	19.0	12.6 29.2	19.4	6.4 29.1	18.4	−1.8 29.4	7.0	−0.3 12.1	9.1	−0.8 21.8	7.1	3.2 13.4	14.2	−0.4 22.1
3 hours			16.7	8.7 26.1	15.1	−0.3 28.0			6.0	0.0 11.5	4.8	1.3 9.5	12.5	−1.6 26.7
Arm														
Basal	33.2	27.0 36.5	33.1	29.9 34.7	33.4	22.4 37.3	32.9	31.1 34.7	32.3	27.4 36.2	33.5	30.5 35.0	33.2	29.2 36.1
1 hour	33.3	29.3 36.8	30.8	27.6 32.0	29.4	14.7 37.4	30.8	27.9 35.7	30.4	21.2 35.8	32.4	29.1 35.8	26.2	19.1 31.2
2 hours	33.0	30.6 36.3	29.5	26.1 31.1	27.6	15.3 34.7	30.8	17.9 27.4	29.3	20.7 35.7	31.3	26.9 35.3	24.3	16.6 31.3
3 hours	33.6	32.4 35.5	27.6	24.0 30.2	26.6	13.0 33.8			31.9	23.2 32.3	30.0	28.3 32.7	22.7	14.8 28.1
Calf														
Basal			31.2	29.7 32.8	31.2	20.5 36.8	32.1	27.9 34.4	32.0	26.9 34.4	32.8	30.5 34.6	32.4	28.9 34.9
1 hour			24.7	21.0 28.4	23.2	12.1 32.2	23.8	21.0 26.9	23.1	18.7 29.7	24.9	21.8 29.7	23.3	18.6 31.0
2 hours			22.6	17.5 28.5	21.2	11.4 31.4	21.6	17.9 25.3	21.2	14.3 26.3	22.9	19.0 28.5	21.1	15.6 28.7
3 hours			21.0	16.7 27.8	20.7	13.0 31.3			20.3	15.6 25.8	22.7	19.4 28.7	20.1	15.4 28.4
Chest														
Basal	33.1	28.3 35.7	34.1	31.9 35.7	34.1	26.4 37.9	34.0	31.4 35.7	33.0	28.2 36.1	34.6	33.4 35.4	34.6	30.3 37.1
1 hour	34.6	31.5 37.0	34.6	31.2 37.8	33.4	23.0 36.7	34.9	30.4 38.2	33.3	27.1 37.6	34.6	31.7 37.6	33.6	17.3 37.8
2 hours	34.7	32.3 36.4	34.0	29.1 38.7	32.9	25.9 36.4	35.3	32.7 36.8	33.4	28.9 37.6	34.7	31.8 37.6	33.4	19.0 36.6
3 hours			34.4	31.0 35.4	32.8	23.1 37.5			33.4	31.0 37.4	33.9	31.9 35.8	33.4	18.9 35.9

cated the attainment of an equilibrium between input and loss of heat from the clothed body. The relative stability of the rectal temperature in this last 1½ to 2 hours of exposure (Table III) adds additional emphasis to this suggested leveling-off phenomenon. With the exception of the curves for environments of − 26.1° and − 34.4° C., there appeared at the end of 3 hours to be a greater drop and a lower final mean skin temperature the colder the environment.

The ranges of skin and rectal temperatures are presented as an indication of the variability to be expected, and the futility of depending upon observations made on a few subjects in experiments

of this nature. The frequency distribution of the mean skin temperature during the 3-hour exposure to 2 of the environments studied, viz., − 17.8°, and − 40.0° C. is shown in Figures 2 and 3. They emphasize the rapidity of drop in mean skin values and the scatter that can be expected. Unfortunately, they fail to show that an individual may start off with one of the higher initial temperatures, but end the period of exposure with one of the lowest. For example, at the − 40° C. environment, one subject had an initial value of 35.3° C. and a final value of 17.2° C. while another started with 35.2° C. and ended with

28.3° C. In these cases, neither subject complained of the cold; only the first subject shivered and this occurred after 170 minutes of exposure.

The data on values obtained for arm, chest thigh, calf, and toe are also given in Table II a additional evidence of variability, and to show th regional differences in rate and degree of chang in the temperatures of these parts. The suscept bility of the extremities to low environmenta temperatures, and the progressively greater in adequacy of foot protection, are indicated by th frequency distribution diagrams of Figures 4 an 5. Toe temperatures below 0° C. were recorded i

TABLE III

Rectal temperatures of men sitting quietly at designated environmental temperatures

Room temperature	Basal		First hour		Second hour		Third hour	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
°C	°C	°C	°C	°C	°C	°C	°C	°C
22.2	37.8	37.4 38.1	37.1	36.1 37.6	37.0	36.4 37.4	37.0	36.3 37.6
1.1	37.4	37.2 37.6	36.9	36.6 37.2	36.8	36.4 37.0		
−17.8	37.4	37.0 37.9	37.2	36.6 38.1	36.9	36.3 37.7	36.7	36.3 37.3
−23.3	37.8	36.9 38.4	37.2	36.7 37.5	36.8	35.9 37.6	36.8	35.8 37.6
−26.1	37.4	37.0 37.8	37.0	36.8 37.4	36.7	36.4 36.9		
−28.9	38.0	36.1 38.2	37.0	36.0 37.6	36.8	35.8 37.2	36.7	35.4 37.1
−34.4	37.3	36.9 37.6	37.1	36.8 37.3	36.6	36.4 36.7	36.4	36.1 36.7
−40.0	37.4	37.0 38.1	36.9	36.8 37.2	36.8	36.2 37.2	36.7	36.1 37.1
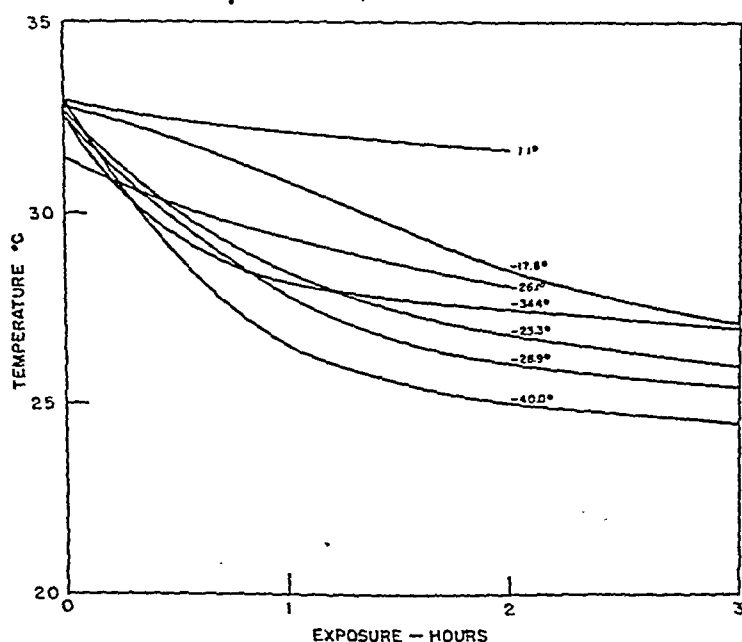
FIG. 1. MEAN SKIN TEMPERATURES OF SITTING MEN EXPOSED TO LOW ENVIRONMENTAL TEMPERATURES

FIG. 3. FREQUENCY DISTRIBUTION IN MEAN SKIN TEMPERATURE OF MEN SITTING DURING A 3-HOUR EXPOSURE TO −40.0° C.

FREQUENCY DISTRIBUTION IN MEAN SKIN TEMPERATURE OF MEN SITTING DURING A THREE-HOUR EXPOSURE TO −17.8° C.

FIG. 5. FREQUENCY DISTRIBUTION IN TOE TEMPERATURE OF MEN SITTING DURING A 3-HOUR EXPOSURE TO −40.0° C.

FIG. 4. FREQUENCY DISTRIBUTION IN TOE TEMPERATURE OF MEN SITTING DURING A 3-HOUR EXPOSURE TO −17.8° C.
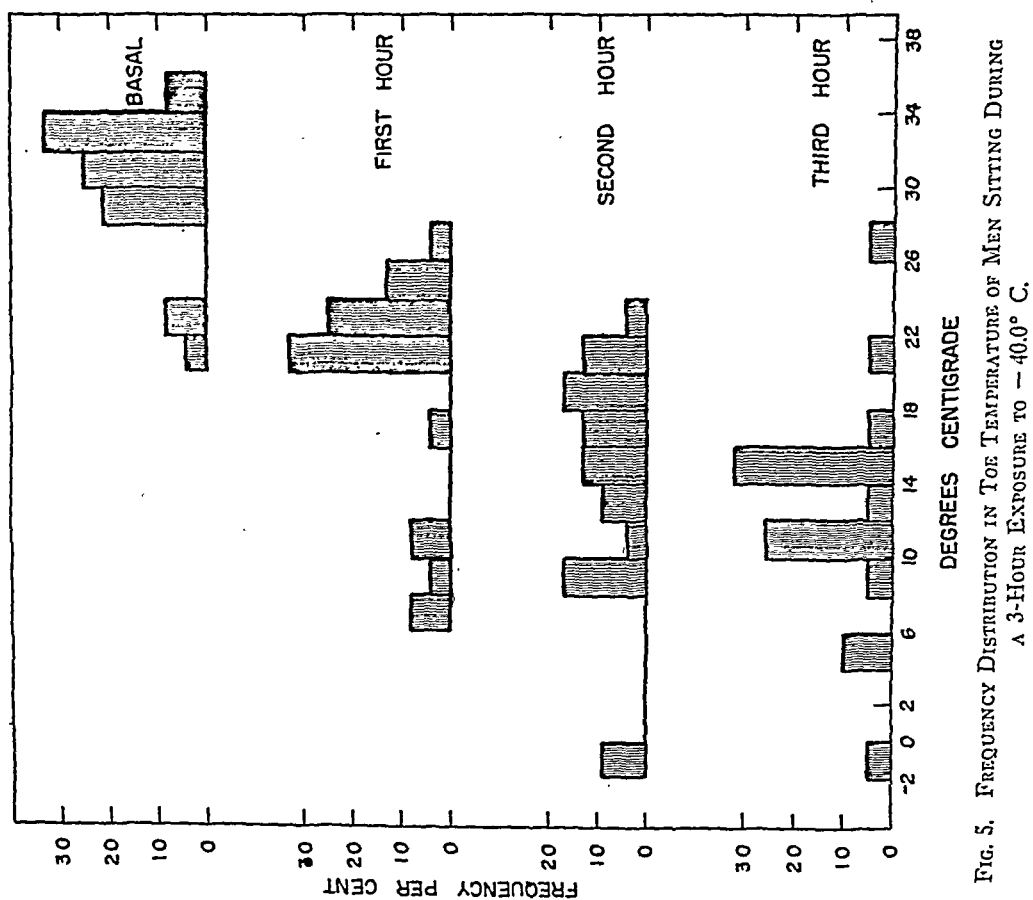
environments of − 23° C. and below. These occurred in a number of cases before 2 hours of the exposure had elapsed. In some individuals a phasic type of response was observed, in which the toe temperature, after the above amount of cooling, suddenly rose 5 to 10 degrees, but returned again very rapidly to lower levels. Other individuals remained at these low levels. No cases of frostbite were observed in any of the subjects. Subjective complaints regarding toes and other areas could not be definitely related to the temperature of the part. Frequently the complaints were more vigorous with the higher temperatures than with the lower.

The observations made on the diversity in rate and extent of fall in mean skin or individual part temperatures, were also observed with rectal temperatures (Table III). Considerable caution must be exercised in the interpretation of any single individual value. An interesting observation was the finding that after the men returned to a comfortable environment, 22° C., their rectal temperatures continued to fall. This is probably a reflection of the continuing transport of cooled blood from the body surface to the body core.

DISCUSSION

The variability observed in the reactions of men to cold environments are related, although not as a positive correlation, to the variations in metabolism, in mean skin, body and in unit area temperatures. Since all subjects wore clothing offering essentially similar insulative protection, and since tests were made on each man in several environments, these findings are of importance in the final evaluation of protective clothing and susceptibility of men to the effects of cold. Most subjects followed a regular pattern in that the colder the environment the more uncomfortable they were, by both subjective and objective criteria. However, a few men were comfortable at lower temperatures and uncomfortable at certain higher ones. The cause of these variations could not be determined, and remains a major stumbling block to a delineation of the physiologic responses of men to cold environments.

On exposure to cold, the total metabolism is increased (Table I). The cause of the increased heat production is not clear, and considerable dispute has arisen as to whether the increase is in-

duced solely through muscular movements, including shivering, or whether other mechanisms are brought into play. Cannon *et al.* (2), favors a humoral factor, *i.e.*, adrenalin, as being the factor, suggesting that increased metabolism is due to the increased secretion of this hormone. Other investigators (3), while favoring the humoral theory, feel that the adrenal cortex plays the more significant role in adaptation of animals to cold environments. Krogh (4) believes that the increased activity of the animal exposed to cold may be the factor increasing muscle tonus at rest, and so affecting the basal rate.

There is no doubt that even adequately clothed individuals shiver, sometimes quite violently, while sitting in the cold, and that the large increases in heat production observed in the last hour to hour and a half in our subjects were mainly due to this activity. It was difficult to explain the increases in the first hour on this basis, as gross shivering was generally absent. Although increased muscular tonus may be the cause, it was impossible by methods employed to determine any evidence of greater tension. The following table is an analysis of subjective data on shivering obtained at an ambient temperature of − 28.9° C.

Number of subjects *per cent*	Exposure time at onset of shivering
10	Under 66 minutes
25	Under 87 minutes
50	Under 119 minutes
75	Under 151 minutes
90	Under 181 minutes

At this environment of − 28.9° C., only a small fraction of the group exhibited even the mildest shivering in the first hour, although the heat production of the group had increased almost 13 per cent.

These data substantiate neither the chemical nor the muscular activity theories of increased metabolism. Alterations in muscular tone cannot be eliminated as a cause of the raised heat production, and since it was impossible in these experiments to demonstrate an increased secretion of epinephrine, the role of this factor cannot be clearly evaluated. However, since the experiments of de Barenne, *et al.* (5) indicated that increased muscle tone is not associated with any high degree of metabolism, the chemical theory is an attractive explanation for the initial increase in the metab-

olism of clothed men sitting quietly in a cold environment. Furthermore, Hicks (6) has performed experiments on the Australian aborigine, suggesting that the increased heat production at low environmental temperatures is not brought about by shivering.

Higher skin temperatures were sometimes associated with high metabolic rates, but the contrary was also observed, i.e., high metabolism with lowered skin temperatures. Swift (7) reported that his partially nude subjects began to shiver when the skin temperature reached 19° C. No such correlation was found in the present observations, but heavy clothing worn by these men may have interfered with their responses. When shivering did occur, skin temperatures of 29° C. to 16° C. were recorded. Individuals had different responses on different days under identical environmental conditions.

The changes in rectal temperature bore no relation to the metabolic rate. Swift's (7) data on lightly clothed subjects indicated that changes in rectal temperatures were not a stimulus to shivering and, therefore, were not related to the increased metabolic rates observed. Vaughn's (8) metabolic studies on subjects whose rectal temperatures had been lowered to approximately 84° F., disclosed that there was a relationship between rectal temperatures and metabolism, since all subjects had a markedly lowered metabolic rate. However, Dill and Forbes (9) in similar experiments reported the total energy exchange to be above basal levels due to shivering, voluntary activity, and a muscular rigidity of unknown origin.

The data that have been presented illustrate some of the physiological changes that occurred in men exposed to low environmental temperatures. The responses of man to cold is complicated by a number of extraneous factors which are, at present, poorly understood. Physiological and psychological studies in progress at the present time will, it is hoped, clarify a number of points regarding the variability of the response of clothed men to very cold environments.

SUMMARY

1. Continuous observations were made of the metabolic rate, skin and rectal temperatures of men while dressed in Artic uniforms and sitting quietly in extremely cold environments. Ambient temperatures ranged from 1.1 to − 40.0° C.

2. The heat production in the cold was above basal values during the entire test period. In the − 40° C. environment, average metabolic increases of 13, 53, and 74 per cent were recorded for the first, second and third hours respectively. The rise in heat output during the first hour could not be explained on the basis of shivering. In the third hour, shivering was present in the majority of the subjects. Neither the role of chemical mediators, nor that of increased muscular tonus, could be clearly delineated, and require additional investigation.

3. The fall in rectal temperatures was moderate, although values of 35.4° C. were occasionally observed. The absolute value was not correlated with the presence of shivering and, therefore, low rectal temperatures could not be considered as the stimulus for shivering.

4. Mean skin temperatures fell precipitously during the first hour of exposure, and were stabilized before the end of the test period. Considerable variability was observed in both the rate and extent of fall, not only in different men, but in repeat tests on the same subject.

5. Of all the skin areas, the hands and feet exhibited the greatest temperature changes in both rate and degree of fall. Toe temperatures below 0° C. were noted in several instances. The susceptibility of the extremities to cold environments was related to their sensitive vasomotor mechanisms, and to the fact that they were provided with the least amount of insulative protection.

6. The responses of men exposed to cold environments are subject to considerable variation, and extreme care must be exercised in the interpretation of data obtained, whether on a few or a large number of subjects.

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EFFECT OF METHEMOGLOBINEMIA ON THE VISUAL THRESHOLD AT SEA LEVEL, AT HIGH ALTITUDES, AND AFTER EXERCISE

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Experiments by one of us (1) have shown that methemoglobinemia exerts a protective effect in dogs against poisoning due to inhalation of HCN and CNCl. The possibility of applying these findings in man raised the question as to the extent to which methemoglobinemia would impair various physiological functions. Several groups of investigators (2 to 4) have found that a rise in the threshold of the dark adapted eye occurs during anoxia induced by low oxygen tensions, and have considered this visual test a very sensitive criterion of this type of anoxia. Accordingly, it was decided to determine whether the anoxia due to methemoglobinemia would affect the visual threshold (a) at sea level, (b) at lowered oxygen tensions, and (c) after exercise.

EXPERIMENTAL

Methemoglobinemia was induced by the ingestion of p-aminopropiophenone. This substance was shown by Vandenbelt, Pfeiffer, Kaiser and Sibert (5) to be a potent methemoglobin-former in animals. Doses ranging from 1 to 2 mgm. per kgm. were dissolved in a minimal volume of propylene glycol, usually not more than 7 ml., and administered by mouth. Propylene glycol, without dissolved p-aminopropiophenone, was also given to a few individuals.

The concentration of methemoglobin was determined by a slight modification of the method of Evelyn and Malloy (6) and expressed as per cent of the total blood pigment. After ingestion of the p-aminopropiophenone in propylene glycol, the concentration of methomoglobin usually rose to a maximal value in about an hour. The concentration then remained fairly constant for about another hour when it began to decrease slowly. Measurements of the threshold were usually made during the period of constancy of the methemoglobin concentration, i.e. 1 to 2 hours after the ingestion of the drug. In the earlier experiments, a sufficient number of blood samples was taken so as to obtain a curve of the change in methemoglobin concentration, and to assure accurate estimation of the methemoglobin concentration at the time of measuring the dark adaptation.

The thresholds were measured with a Hecht-Shlaer adaptometer, Model 3. This instrument has a 3° blue test field which appears 7° below a red fixation point. The test field is exposed in flashes of 0.2 second, accurately controlled by a pendulum shutter. The subject is seated comfortably at the instrument and views the test field binocularly with the natural pupil. The device for controlling the shutter and exposing the field is easily accesible to the subject. The experimenter is seated directly across from the subject, at the other side of the instrument, where he can regulate and note the brightness of the test field. At a signal from the experimenter, the subject operates the shutter and informs the experimenter whether or not he has been able to discern the exposed field. The general principles and technique of dark adaptation measurements have been discussed by Hecht and Shlaer in describing their Model 1 adaptometer (7).

The measurements of the thresholds were expressed as log micromicrolamberts. The absolute values of these measurements are not relevant to the present problem and are not recorded here. The changes in the thresholds are expressed in the present paper in logarithmic units. Changes of 0.1, 0.2, 0.3 log units correspond, respectively, to changes of 26, 59 and 100 per cent in the threshold.

To avoid exposure to sunlight en route to the laboratory, the subjects wore dark red goggles. The subjects were then kept in a dark room for at least 30 minutes before the visual threshold was determined. p-Aminopropiophenone was then administered as described above. A blood sample was taken at the end of about an hour; during this procedure, the subject's eyes were covered, or a dim red light was used, while the blood was being taken. The visual threshold was again determined until it was constant, and another blood sample taken.

In those experiments in which the effect of lowered oxygen tension was determined, the subject was dark adapted and the threshold determined until a constant value was obtained. A nose oxygen mask was then adjusted and the threshold determined in a flow of 20 per cent oxygen. A 13 per cent oxygen mixture was then switched in for 15 minutes and the threshold determined at the end of this time. In most instances, the subject was then allowed to breathe room air for about 3 minutes; this was followed by 15 minutes of 10 per cent oxygen unless the subject became distressed. Occasionally, the subject was transferred directly from a 13 per cent to a 10 per cent oxygen mixture. The visual threshold was determined at this time, and again after the subject had been returned to room air.

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This entire procedure was repeated after the induction of methemoglobinemia.

The effect of exercise on dark adaptation was determined in 4 trained and 8 untrained subjects, as follows: The subjects were dark adapted and the visual thresholds determined. They then mounted the cycle ergometer and performed usually 3, and in some instances 5, minutes of exercise at various loads. Records of these loads were kept, but no correlation was found between the magnitudes of these loads or the degree of training and the visual effects. The work done ranged from about 20,000 foot pounds for the untrained individuals, to about 30,000 to 35,000 foot pounds for the trained individuals. The thresholds were determined within 2 minutes after the end of the exercise bout, and in most instances, several times again within the next 10 to 20 minutes. Some of the individuals received this exercise test several times, each time on a different day. When the effect of methemoglobinemia was to be determined, p-aminopropiophenone was administered immediately after the first post-exercise threshold determination. To save time, and since it was known on the basis of previous experience that the concentration of methemoglobin was negligible during the first 5 to 10 minutes after administration of the drug, several threshold determinations were made during the period to ascertain the effect of the bout of exercise during the non-methemoglobinemic period. The subjects then donned red goggles and were allowed to leave the dark room for about 30 to 45 minutes. They returned to the dark room and remained there for about 15 minutes before threshold measurements were begun. A blood sample for determination of methemoglobin concentration was taken just before exercise, when, in accordance with previous experience, the concentration was maximal and constant. The threshold was determined within 2 minutes after exercise and again 1 or more times during the course of the next 15 to 20 minutes.

RESULTS

Effect of methemoglobinemia on visual threshold at sea level. Sixty-four determinations of the effect of methemoglobinemia on the visual threshold at sea level were made in 32 subjects at various times and in connection with the several experiments. These included 3 determinations in which propylene glycol without dissolved p-aminopropiophenone was administered, and 2 determinations in which, although p-aminopropiophenone was apparently taken, no methemoglobinemia resulted. The changes in the threshold are shown in Figure 1.

Inspection of Figure 1 shows a preponderance of increases in threshold, but no apparent correlation of increase in threshold with degree of methemoglobinemia. Detailed analysis shows that the average or mean increase, excluding the 5 values
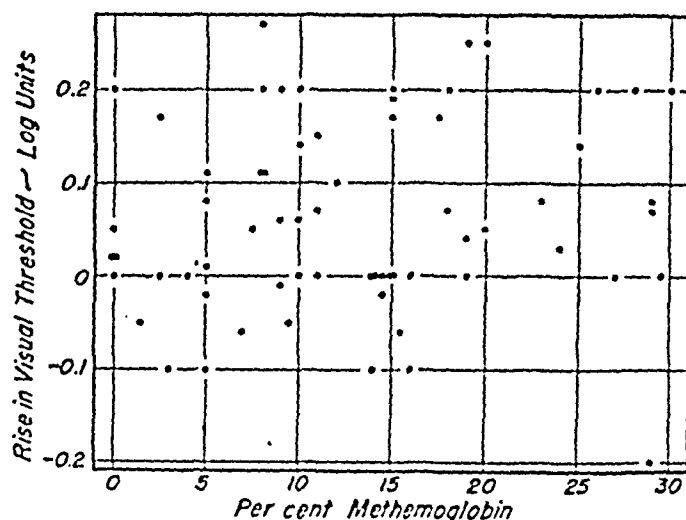
FIG. 1. EFFECT OF METHEMOGLOBINEMIA ON THE VISUAL THRESHOLD OF THE DARK ADAPTED EYE

in subjects in whom no methemoglobin was formed, was 0.063 log units ($t = 4.5$; $P = < 0.01$). Apparently, this increase was significant. However, comparison of this change with those observed in 5 men receiving propylene glycol without p-aminopropiophenone and subjected to the same procedure of veni-puncture, etc., indicated that this increase was of the same order of magnitude and could not be ascribed to the methemoglobinemia *per se*. Moreover, correlation of the increase in the visual threshold with the degree of methemoglobinemia for all the 64 determinations gave a correlation coefficient, r, equal to 0.108. The probability that this value might arise by chance is greater than 0.1. It may be concluded, therefore, that there was no significant increase in the visual threshold at sea level in individuals having concentrations up to 30 per cent methemoglobin.

Effect of methemoglobinemia on visual thresholds at simulated high altitudes. The visual threshold was determined in a series of men breathing 13 per cent oxygen (12,000 feet) and 10 per cent oxygen (18,000 feet) before and after the induction of various degrees of methemoglobinemia (Table I). It may be seen that before the induction of methemoglobinemia, the average rise in the threshold was 0.13 log units at 13 per cent oxygen, and 0.51 log units at 10 per cent oxygen. Statistical evaluation gave respectively, t values of 2.7 ($P = 0.03$) and 5.3 ($P = < 0.01$). These increases are significant and in good agreement with those previously reported by other workers

TABLE I

*Effect of methemoglobinemia on visual thresholds
at simulated high altitudes*

Name	Increase in threshold		Increase in threshold in methemoglobinemia	Concen. of methemoglobin
	Without methemoglobinemia	With methemoglobinemia		
	log units	*log units*	*log units*	*per cent*

a. At 13 per cent oxygen (12,000 ft.)

E.A.	0.12	0.22	0.10	0
J.B.	0.20	0.35	0.15	1
H.S.	0.55	0.60	0.05	2
W.W.	0.12	0.25	0.13	2
J.S.	0.13	0.15	0.02	5
J.T.	0.10	0.10	0.00	8
W. Mc.	−0.04	0.05	0.09	9
R.K.	0.19	0.11	−0.08	15
D.S.	0.04	0.28	0.24	19
D.S.	0.04	0.50	0.46	20
G.G.	0.00	−0.02	−0.02	23
E.S.	−0.02	0.05	0.07	24
Average	0.13		0.10	
t value	2.7		2.45	
P value	0.03		0.04	

b. At 10 percent oxygen (18,000 ft.)

J.B.	0.85	0.85	0.00	1
W.W.	0.92	0.75	−0.17	2
H.S.	0.35	0.30	−0.05	3
W. Mc.	0.56	0.85	0.29	8
J.T.	0.47	0.68	0.21	10
R.K.	0.49	0.31	−0.18	15
E.S.	1.45	0.70	−0.75	15
W. Mc.	0.20	0.30	0.10	18
W.T.	0.50	0.70	0.20	18
D.S.	0.24	0.60	0.36	20
D.S.	0.24	0.48	0.24	20
J.McF.	0.30	0.51	0.21	21
R.K.	0.23	0.83	0.60	29
D.S.	0.22	0.60	0.38	30
Average	0.51		0.10	
t value	5.3		1.18	
P value	<0.01		>0.2	

for the effect of altitude on dark adaptation (2 to 4).

Table I also shows the changes in the rod threshold resulting from the induction of various degrees of methemoglobinemia at 13 per cent and 10 per cent oxygen mixtures. At a 13 per cent oxygen mixture, the rises in the visual threshold after the *induction of* methemoglobinemia averaged 0.10 log unit higher than prior to such induction. This difference was just significant ($t = 2.45$; $P = 0.04$). At a 10 per cent oxygen mixture, the difference between the rises in the visual threshold before and after induction of methemoglobinemia was 0.10 log unit

($t = 1.18$; $P = > 0.2$). Exclusion of the 2 extreme values, − 0.75 for E. S. and 0.60 for R. K., gave a rise of 0.13 ($t = 2.37$; $P = 0.04$).

These increases in the visual thresholds as the result of induced methemoglobinemia appear to border on significance. However, it should be noted that here, as in the experiments at sea level, the induction of methemoglobinemia of whatever degree, was accomplished by the drinking of a solution, and accompanied by 1 or more venipunctures, procedures to which the non-methemoglobinemic individuals were not subjected. Correlation of the increase in the visual thresholds with the degree of methemoglobinemia gave coefficients of 0.163 with a P value of > 0.1 for the experiments at 13 per cent oxygen, and of 0.483 with a P value of 0.07 for those with 10 per cent oxygen. In view of these considerations and the small number of cases, there is no evidence for concluding that methemoglobinemias ranging up to 30 per cent significantly alter the visual thresholds at simulated altitudes of 12,000 and 18,000 feet.

Effect of exercise on the visual threshold in non-methemoglobinemic and methemoglobinemic individuals. Sixteen determinations were carried out on 8 non-methemoglobinemic individuals before and within 1 to 2 minutes after exercise, as previously described. Table II shows that there was

TABLE II

*Changes in visual threshold in non-methemoglobinemic
individuals immediately after short bout of exercise*

Name	Increase in rod threshold
	log units
D.B.	0.10
	0.20
	0.10
	−0.30
H.	0.00
	−0.05
O.	0.10
	−0.10
B.S.	0.20
	−0.20
	0.00
	0.10
C.H.	0.00
G.	0.00
T.	0.10
B.	−0.15
Average	0.006

Duration of exercise 3 minutes in all but 4 experiments which were 5 minutes. Total amount of work ranged from 17,200 foot pounds to 37,200 foot pounds; lower amounts were done by untrained men, higher amounts by trained men.

tensions (simulated high altitudes) are additive. In the present study, it has been found that methemoglobinemia does not alter the threshold in rod adaptation, either at sea level or at high altitudes.

The possibility exists that the anoxia of carboxyhemoglobinemia or methemoglobinemia raises the threshold of the cones but not that of the rods. There are, however, no existent data to lend support to this possibility. In anoxia due to low oxygen tensions, the shape of the dark adaptation curve is the same; the whole curve is shifted to a higher level, and both the cone and rod thresholds are raised to the same extent (9).

An attempt to explain the differences in effect on sensitivity to light in methemoglobinemia and carboxyhemoglobinemia may also be made by considering the comparative degrees of tissue hypoxia in these 2 conditions up to levels of about 30 per cent carboxyhemoglobinemia or methemoglobinemia. Adjustments in the oxygen unloading in the tissues up to this level of carboxyhemoglobinemia are accomplished not by changes in cardiac output, but by decreases in the venous oxygen tension (10, 11). Both methemoglobinemia and carboxyhemoglobinemia shift the oxygen dissociation curve of the residual oxyhemoglobin to the left, and render the curve less sigmoid and more hyperbolic. This shift is less marked in the case of methemoglobinemia (12, 13). Therefore, for a given amount of oxygen unloading, a lower venous and tissue oxygen tension is reached in the case of carboxyhemoglobinemia than in the case of an equivalent degree of methemoglobinemia.[2] In other words, at equivalent levels of

methemoglobinemia and carboxyhemoglobinemia, tissues should be more sensitive to the anoxia of the latter. This may explain why dark adaptation may be more readily impaired in carboxyhemoglobinemia (8) than in methemoglobinemia. However, there are not enough data to permit a quantitative evaluation of the relative impairments in these 2 types of anoxia.

Of considerable interest is the finding that the threshold was affected by exercise. In nonmethemoglobinemic individuals, the threshold was not altered within 1 to 2 minutes after conclusion of the exercise, but was significantly higher 5 to 10 minutes after the end of exercise. In contrast, methemoglobinemic individuals showed a *decrease* in the threshold immediately after the end of exercise. About 5 to 10 minutes later, the threshold rose not only enough to negate this decrease, but also above the normal level, to an extent equal to that found in the non-methemoglobinemic individuals.

The changes in acid base equilibrium following short bouts of severe exercise were studied in considerable detail by Barr and Himwich (16). The amounts of work done ranged from 3500 to 4000 kgm. meters (equivalent to 25,000 to 30,000 foot pounds) in 3½ minutes, and therefore corresponded to the work loads in the present experiments. The CO_2 capacity of the arterial blood was diminished during the second minute of exercise, and became progressively lower during the exertion and for several minutes (3 or more) after the exercise had ended. The return to normal CO_2 capacity was gradual. The decreases in pH paralleled, in general, these decreases in CO_2 capacity.

According to Wald, *et al* (3), acidosis causes a rise in the rod threshold, whereas alkalosis causes a decrease. The rise in rod threshold, both in methemoglobinemic and non-methemoglobinemic individuals, 5 to 10 minutes after exercise, may therefore be explained in terms of the acidosis of the post-exercise period. The decrease in the methemoglobinemic individuals immediately fol-

[2] This may be illustrated by a consideration of Roughton's and Darling's oxygen dissociation curves in the presence of carboxyhemoglobinemia (14) and by the findings that about twice as much methemoglobinemia as carboxyhemoglobinemia is necessary to produce the same shift in the oxygen dissociation curve of the residual hemoglobin (12, 13). From Figure 1 of Roughton and Darling (14), the oxygen delivery in absence of any carboxyhemoglobinemia or methemoglobinemia between an arterial tension of 100 mm. and a venous tension of 40 mm. will be (96 per cent − 74 per cent) × 20 vols. per 100 ml., or 4.4 vols. per 100 ml. The same oxygen delivery in the presence of 40 per cent carboxyhemoglobin, would be (96 per cent − 59.3 per cent) × 12 vols. per 100 ml. The venous and tissue oxygen tension at a venous saturation of 59.3 per cent would be 19 mm. Since a 40 per cent methemoglobinemia may be considered to produce approximately the same shift as a 20 per cent

carboxyhemoglobinemia (13 to 15), an unloading of 4.4 vols. oxygen per 100 ml. would be accomplished by going from a 96 per cent saturation at 100 mm. arterial tension to 59.3 per cent at 24 mm. venous tension. Thus at equivalent degrees of carboxyhemoglobinemia and methemoglobinemia, the venous and tissue oxygen tensions are lower in carboxyhemoglobinemia.

lowing exercise, and the absence of such a decrease in non-methemoglobinemic individuals, do not agree with such an explanation. However, the arterial oxygen content (17) and the cardiac rate are increased immediately following a severe short bout of exercise, and the latter falls rapidly within 2 minutes after the end of exercise (18). Although it is possible that these factors influence the visual threshold and do so differently in the presence and absence of methemoglobin, data in the literature are inadequate to evaluate this influence.

SUMMARY

1. Concentrations of methemoglobin up to 30 per cent were induced without showing any definitely significant effect upon the rod threshold in dark adaptation either at sea level or at simulated high altitudes of 12,000 feet and 18,000 feet.

2. Short bouts of severe exercise lower the rod threshold immediately after exercise in methemoglobinemic individuals, but not in normal individuals. However, 5 to 10 minutes after exercise, the threshold rises above the normal threshold to the same extent in both methemoglobinemic and non-methemoglobinemic individuals.

3. The above findings are discussed with reference to oxygen unloading in tissues, and to the effect of exercise on the acid-base balance.

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VOLUNTARY BREATHHOLDING. I. PULMONARY GAS EXCHANGE DURING BREATHHOLDING [1]

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INTRODUCTION

When a subject is totally immersed in water while holding his breath, a loss of buoyancy occurs. This phenomenon can be demonstrated by resting the subject on 1 pan of a balance, the loss of buoyancy being reflected as a gain in weight on the balance. This gain in weight (or loss of buoyancy) was first observed by us while determining body density by an underwater weighing method (1). This gain in weight amounts to 200 to 500 grams per minute, depending on the size of the subject, and is due to a decrease in the volume of gas in the lungs during breathholding.

This report presents data describing this phenomenon and indicating its significance with respect to pulmonary gas exchange during breathholding.

METHODS

The apparatus for underwater weighing consists of a metal rectangular tub 7 feet by 2½ feet by 2 feet. Suspended in this tub is a pan on which a person can lie. The pan is suspended on a balance with an index scale of 500 grams graduated to 1 gram, and with a capacity of 5 kgm. A vertical tub is also used to advantage for some purposes, but data in this report are from experiments in the horizontal tub, in which pressure on the lungs and abdomen is similar, and amounts to only a few inches of water pressure. The subject is first tested under water so that the pan is properly balanced longitudinally and laterally, and sufficient counter weights are added so that the total weight is about 2 kgm. greater than that of the water displaced. The subject, after taking a deep breath, submerges and rests quietly on the pan while consecutive weighings are recorded as long as he can hold his breath. In experiments reported here, the usual preparation for breathholding was for the subject to expire maximally, and then inspire maximally,

after breathing room air, commercial oxygen (approximately 100 per cent) or 10 per cent oxygen—90 per cent nitrogen for 3 to 5 minutes.

In order to learn more concerning CO_2 and oxygen exchange during breathholding, studies of arterial blood gases and pH, and of urinary excretion of CO_2 were carried out during breathholding. The lung volume changes were verified by measuring the vital capacity (volume of maximal expiration) in routine fashion, and comparing it with the volume of maximal expiration at the end of periods of breathholding.

RESULTS

1. Underwater weight change during breathholding. In 60 underwater weighing experiments in which the subjects inhaled 100 per cent oxygen, there was, during breathholding, a consistent steady gain in weight of the range of 200 to 500 grams per minute in each subject, the magnitude varying chiefly with the size of the subjects.

In each of a few subjects, a comparison was made of the underwater weight change following inhalation of 100 per cent oxygen, of room air, and of 10 per cent oxygen. Because of the short period of breathholding which obtains with 10 per cent oxygen, difficulty is encountered in stabilizing the subject on the balance, so that swinging of the balance prevents accurate weighing. In a few instances, accurate weights were obtained with 10 per cent oxygen. A typical example of underwater weight change with each gas mixture is presented in Figure 1.

After breathing 100 per cent oxygen, the change in weight proceeds at a nearly constant rate as long as the subjects can hold their breath, that is, up to 5 to 6 minutes. After breathing ambient air, the change in weight also proceeds at a constant rate, but more slowly than following 100 per cent oxygen. The comparison of rates after 100 per cent oxygen, and after air, is presented in Table I. After breathing 10 per cent oxygen, the weight changes at a comparatively
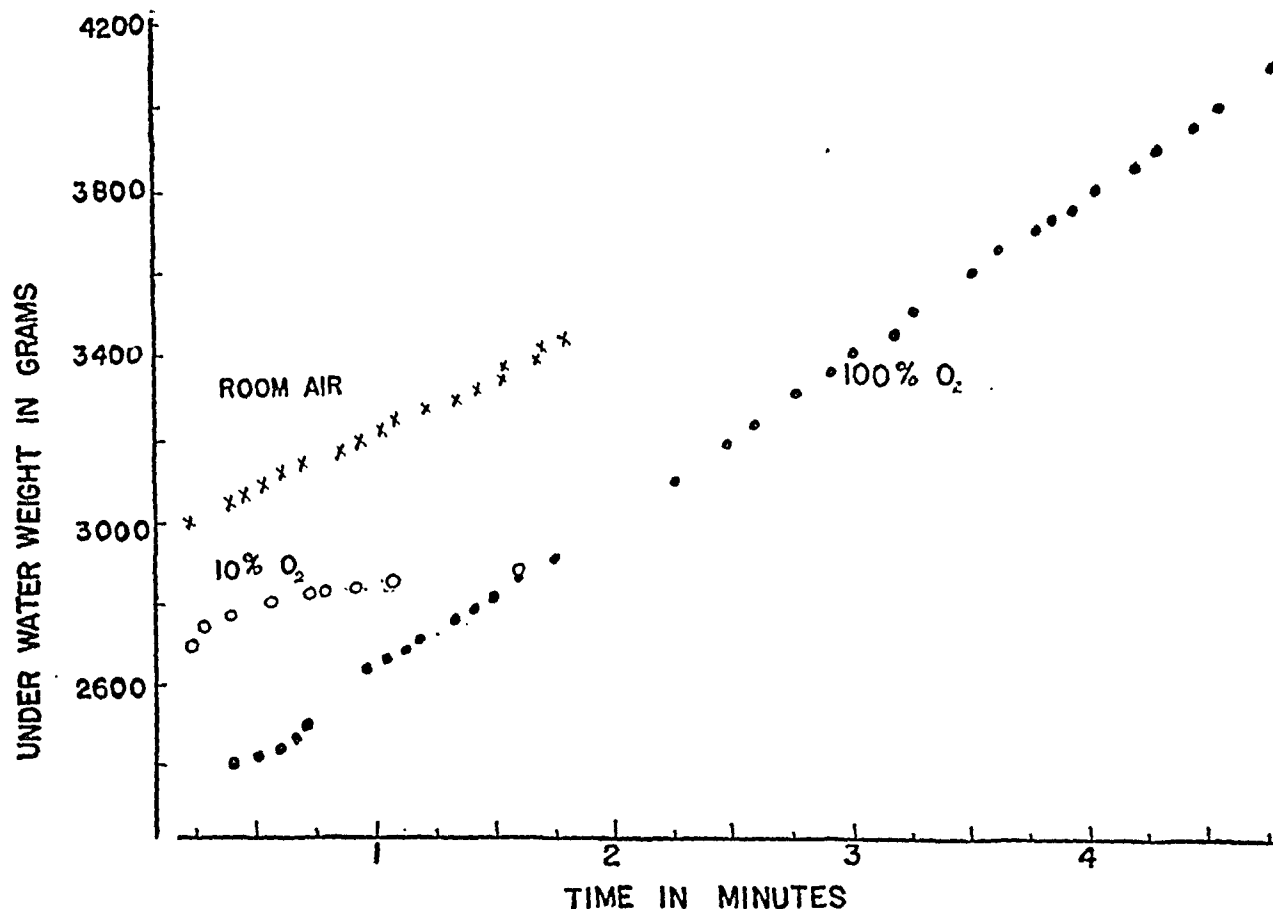
FIG. 1. AN EXAMPLE OF THE CHANGES IN UNDERWATER WEIGHT

These studies made during periods of breathholding immediately following the breathing of air, 100 per cent oxygen, and a 10 per cent oxygen—90 per cent nitrogen gas mixture.

slow rate. These differences in weight gain will be discussed subsequently.

During the underwater weighing, there is no obvious loss of body gas externally, and no apparent reason to assume significant change of gas volume in the intestinal tract. Likewise, the depth of the body underwater is constant, so that changes in the volume of body gas due to variations in external pressure are insignificant. The only way to account for the change in body weight is to assume that the volume of gas in the lungs is decreasing.

2. Changes in vital capacity (maximum expiratory volume) during breathholding. In order to be certain that the above-mentioned phenomenon results from a decrease in the volume of lung gas, and not from an artefact inherent in the underwater weighing, maximum expiratory volume was measured after varying periods of breathholding. The subjects were seated and the vital capacity (maximum expiratory volume) measured after exhaling and then inhaling maxi-

mally. Following adequate training, the subject after inhaling maximally, held his breath for as long as desired and then exhaled maximally into a spirometer. The procedure was carried out with 100 per cent oxygen. Sampling times were arranged to distribute in randomized fashion the variations due to training, fatigue, etc.

An example of the results is presented in Figure 2. The vital capacity decreases as the breath is held, and the rate of change is of the same order of magnitude as that occurring under water. This indicates clearly that the observed loss of weight during underwater breathholding is due to loss of gas from the lungs.

3. The relation of the underwater weight gain to the total oxygen consumption. The rate of oxygen consumption was determined in 3 subjects with a Benedict-Roth metabolism apparatus during inhalation of 100 per cent oxygen. This procedure was either followed or preceded by an underwater weighing following a period of 100 per cent oxygen breathing. The results are pre-

TABLE I

Comparison in the rate of change in underwater weight during breathholding after breathing: A, room air, and B, 100 percent oxygen

Weight Changes

Subject	A		B	
	Run no.	After breathing air	Run no.	After breathing 100 per cent oxygen
		grams per min.		grams per min.
J.K.	1	333	4	365
	2	320	5	355
	3	285		
E.B.F.	8	240	9	330
	11	340	10	340
C.D.S.	1	275	9	410
	2	280	15	295
	3	275		
C.G.	2	250	3	445
	4	285	6	404
	8	275	10	380
I.G. (first series)	1	280	4	370
	3	300	5	340
	9	235	8	330
I.G. (second series)	1	320	2	390
	3	320	4	425
	6	325	5	405
	8	300	7	395
	9	305	10	320
	12	275	11	335
Average		291		369

sented in Table II. The rate of change in gas volume under water was significantly slower than the rate of oxygen uptake as measured by the metabolism apparatus, but the difference was small. If the decrease in weight we observe is due to loss of oxygen from the lungs, it is counteracted by only small accumulations of gaseous CO_2 in the lungs, as, indeed, Hill and Flack found to be the case (2).

4. Loss of carbon dioxide in the urine during breathholding. It is clear that during breathholding only small amounts of CO_2 enter the lungs as a gas. In order to discover whether CO_2 in significant amounts might escape from the body dissolved in urine, a patient was subjected to breathholding with ureteral catheters in place, the urine being collected at 30-second intervals. By means of an intravenous dye test, it was determined that no urine leaked into the bladder around the catheters. The results are shown

in Figure 3. Although some lag occurs between the breathholding and the urinary changes, there is a minor increase in the volume of urine and of CO_2 excreted following breathholding. Taking this lag into account, the maximum loss of CO_2 in the urine possible for the period of breathholding is only 1.2 ml. per minute in the first trial, and 2.0 ml. per minute in the second trial. If total CO_2 production is assumed to be approximately 250 ml. per minute, the loss of CO_2 in the urine accounts for less than 1 per cent of the total.

5. Changes in oxygen content, carbon dioxide content and pH of arterial blood during breathholding. An example of the changes in the gas content of arterial blood during breathholding after maximal inhalation of room air, of 100 per cent oxygen and of ambient air at 16,000 feet, is shown in Figure 4 (also see Voluntary Breathholding, Part III). The initial values are from blood obtained during normal respiration just prior to the breathholding procedure. The immediate sharp fall in CO_2 illustrates the extent to which the single preliminary exhalation and maximum inhalation ventilate the alveoli. If one assumes that the CO_2 content of venous blood rises as does that of arterial blood, there is an accumulation of CO_2 in the blood of, roughly, 2 volumes per cent per minute, or 100 ml. per minute for a circulating blood volume of 5 liters.

The tension of CO_2 (pCO_2) in the arterial blood can be assumed to approximate that in the alveoli. From the increase in alveolar pCO_2 (cal-

TABLE II

Comparison of the rate of oxygen consumption with the rate of change in gas volume during underwater weighing

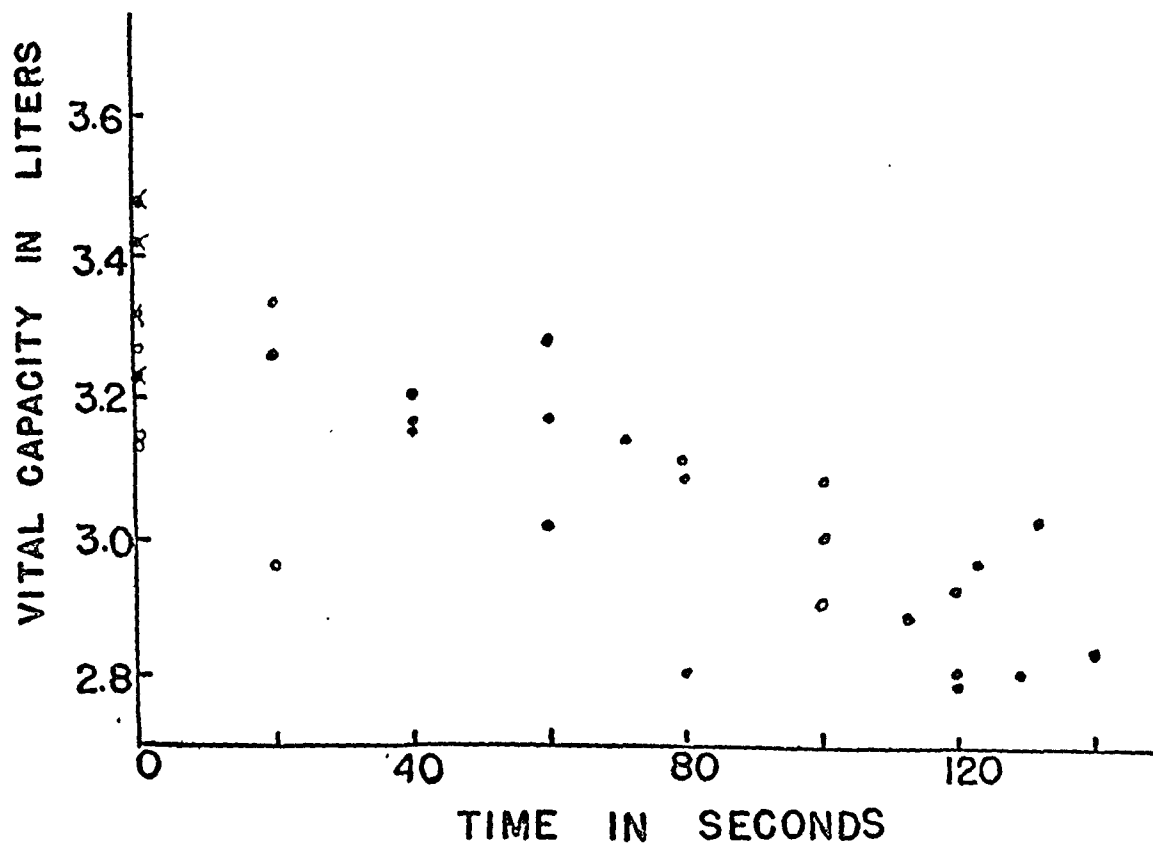| Subject	A Oxygen uptake by the Benedict-Roth Apparatus	B Gas volume change by underwater weighing	Difference between A and B	Remarks
	ml. at N.T.P. per min.	ml. at N.T.P. per min.		
C. G.	339	315	24	B before A
	323	306	17	A before B
I. G.	292	268	24	B before A
	288	264	24	A before B
C. D.	267	240	27	A before B
Mean	302	Mean 279	Mean 23.2	

FIG. 2. THE EFFECT OF VARYING PERIODS OF BREATHHOLDING UPON THE VITAL CAPACITY
(MAXIMUM EXPIRATORY CAPACITY)

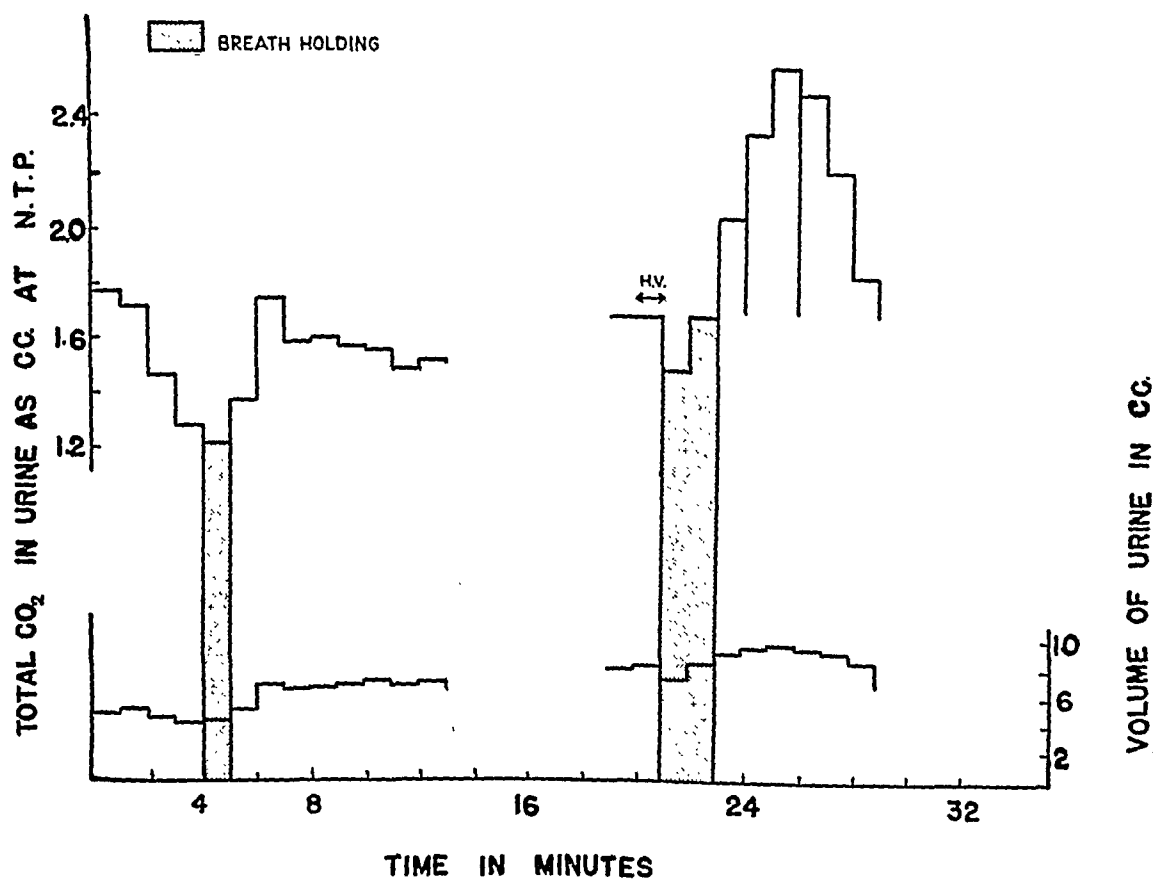Breathholding preceded by breathing of 100 per cent oxygen.

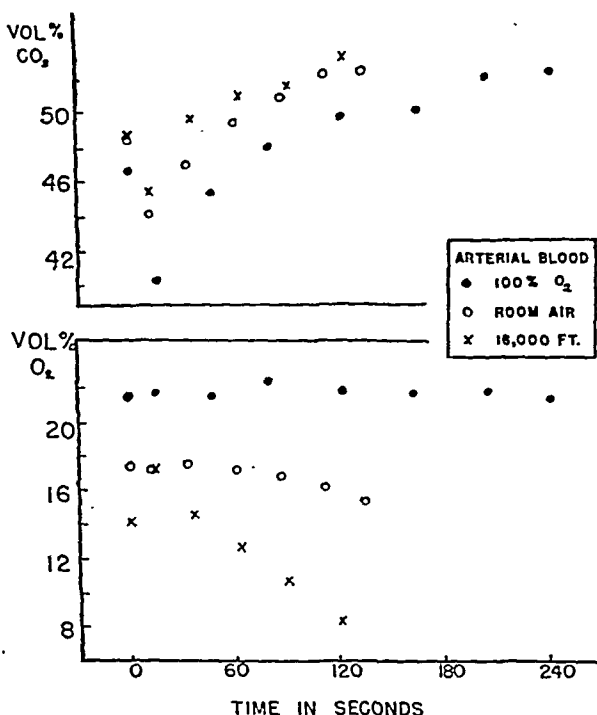FIG. 3. EXCRETION OF CO_2 AND WATER BY THE KIDNEYS DURING BREATHHOLDING

FIG. 4. CHANGES IN THE BLOOD GASES DURING
PERIODS OF BREATHHOLDING

These studies made immediately following the breathing of room air, 100 per cent oxygen, and ambient air at a simulated altitude of 16,000 feet.

culated from CO_2 content and pH of arterial blood) during breathholding, which amounts to little more than 0.5 per cent by volume of CO_2 per minute, one may also conclude that most of the CO_2 being produced by the body remains in a combined state in the blood and tissues, and that very little passes into the lungs in the gaseous state.

The arterial oxygen content after inhalation of 100 per cent oxygen showed no significant change during periods of breathholding as long as 4 minutes. After inhalation of room air, the oxygen content of the arterial blood dropped appreciably during breathholding. And after inhalation of ambient air at 16,000 feet, the oxygen content of arterial blood decreased markedly during breathholding. This will be further exemplified and discussed in the third paper of this series.

DISCUSSION

It is clear, from the data presented above, that there is a marked decrease in the volume of gas in the lungs during breathholding, provided one

has been breathing gas containing 21 to 100 per cent oxygen. It is also apparent that the rate at which this decrease in gas volume occurs, approximates the rate at which oxygen is absorbed from the lungs. Thus, the rate of oxygen uptake of the body as measured by the metabolism apparatus is only slightly greater than the rate of decrease in buoyancy under water following breathing of 100 per cent oxygen. Breathholding with 10 per cent oxygen is accompanied by a very slow rate of buoyancy change. This slow rate may be attributed to the failure of sufficient oxygen to move from the larger gas spaces in the lungs, which might be termed collectively the lung dead space, into the alveoli and the arterial blood. This failure is also demonstrated by the slight decrease in oxygen content of the arterial blood during breathholding with air at ground level, as compared to the marked decrease at a stimulated altitude of 16,000 feet.

The decrease in pulmonary oxygen volume during breathholding is partially counteracted by a slow addition of CO_2 to the gas phase. The results of Hill and Flack (2) demonstrated that increase in alveolar CO_2 is not great during breathholding, their data indicating that 7 to 8 per cent CO_2 by volume might represent the maximum concentration one might expect to find in the alveoli at the end of breathholding following 100 per cent oxygen inhalation. The arterial CO_2 reached in our studies indicate alveolar levels of approximately these percentages, which would give only a small increase in gas volume compared to the decrease due to oxygen absorption. The rate of CO_2 accumulation in the arterial blood during breathholding did not vary at the different oxygen tensions (pO_2) we employed. The reason the CO_2 fails to enter the gas phase in the lungs in greater amounts, is that most of it remains dissolved in the blood and tissues.

SUMMARY AND CONCLUSIONS

1. During underwater breathholding, the buoyancy of an individual as determined by changes in underwater weight decreases progressively. It has been demonstrated that this loss of buoyancy is due to a diminution in lung volume which occurs during breathholding. The decrease in lung volume occurs because oxygen diffuses out of

the lungs much faster than CO_2 diffuses in, most of the CO_2 being retained by solution in the blood and tissue fluid during breathholding.

2. The rate of change in lung volume is dependent upon the pO_2 of the initially inspired air, in that the higher the pO_2, the greater is the rate of loss of lung volume (chiefly oxygen). After inhalation of 100 per cent oxygen, the rate of change in lung volume is only slightly less than the oxygen consumption of the body.

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VOLUNTARY BREATHHOLDING. II. THE RELATION OF THE MAXIMUM TIME OF BREATHHOLDING TO THE OXYGEN TENSION OF THE INSPIRED AIR [1]

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The striking effect of 100 per cent oxygen in lengthening the period of voluntary breathholding became apparent to us during experiments concerned with underwater weighing, in which it was desirable for the subjects to remain immersed as long as possible. Although a few observers (1, 2) have noted this effect of oxygen previously, it has received very little attention in the literature since World War I; and some, notably Schneider (3), concluded that oxygen had a relatively insignificant influence upon the breathholding time.

The studies reported herewith were originally presented (4) as a method for demonstrating objective physiological effects of relatively small changes in altitude (pO_2, oxygen tension). This seemed important, because most functional tests of anoxemia do not reveal changes until altitudes of 12,000 to 16,000 feet are reached. These studies show conclusively that a close relation does exist between variations in maximum voluntary breathholding time and those of the pO_2 of inspired air. This relationship is of interest, because it offers a simple quantitative functional test which is sensitive to relatively slight changes in pO_2 of inspired air, and also because it throws considerable light on the interrelation of oxygen and CO_2 as stimulants which force the subject to start breathing after a period of breathholding.

METHODS

The subjects used in these studies were 40 normal, healthy males, all medical students, with the exception of 4 members of the laboratory team; the mean age of the group was 24 years. The breathholding tests were run on 3 groups of subjects under the following conditions: (1) Ground (746 mm. Hg), 7,000 feet (586 mm. Hg),

10,000 feet (523 mm. Hg), 13,000 feet (464 mm. Hg), and 16,000 feet (412 mm. Hg) breathing ambient air; (2) 16,000 feet (412 mm. Hg), 35,000 feet (179 mm. Hg), 39,000 feet (148 mm. Hg), and 42,500 feet (125 mm. Hg) breathing commercial oxygen (99.3 to 99.7 per cent oxygen) via A–14 demand masks adapted for constant flow; and (3) Ground, breathing compressed air, and oxygen-nitrogen mixtures containing 9.75 per cent, 20.95 per cent, 34.6 per cent, 77.9 per cent and 99.5 per cent oxygen, respectively, as determined by analysis in the Haldane apparatus with mixtures containing less than 50 per cent oxygen, and in the Van Slyke-Neill apparatus with mixtures containing more than 50 per cent oxygen.

The first 2 groups of runs were carried out in the decompression chamber, which was constantly and vigorously ventilated in order to prevent accumulation of CO_2 or oxygen. On all runs the technic of breathholding was as follows: the subjects, in the sitting position, were instructed to make a maximum exhalation, followed by a maximum inhalation, and then to hold the breath as long as possible. Time was started at the end of inspiration, and ended at the beginning of forced breathing. As far as possible, the subjects were kept in ignorance of the prevailing conditions; during the chamber runs the altimeter was covered so that, although aware of changes in altitude in an upward or downward direction, they did not know the exact altitude. In the ground level runs, the tanks containing the gas mixtures were so situated that their labels could not be read by the subject. At least 5 minutes of preliminary breathing was carried out for each exposure. The replicate determinations were never consecutive, and the order of testing was randomized. Duplicate determinations were carried out for each condition tested. There was some tendency on the part of the individual subjects to improve their breathholding ability during the course of the runs. The effect of this was partially offset by giving the subjects several preliminary trials on the ground in order to bring them to their maximum performance, and further controlled by the randomization of the order of testing. With these controlling factors, namely, randomization of testing, it can be assumed that the amount of gas held in the lungs by the individual subject was relatively constant on each test, and that the average value for a number of subjects gave a measure of the maximum breathholding time for a given amount of gas of given composition in the lungs.

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RESULTS

The results are shown in Tables I to III. As expected, there was considerable individual variation in breathholding time; i.e., from 61 to 167 seconds with air at ground level, from 128 to 266 seconds with 100 per cent oxygen at ground level, and from 27 to 83 seconds with air at 16,000 feet. However, each individual yielded surprisingly consistent results, once having achieved proficiency in the procedure.

The duration of breathholding varied directly with the pO_2 of inspired air (corrected for saturation with water vapor at 37° C.) although there were occasional individual inconsistencies; i.e., where a subject did better at 10,000 feet than at 7000 feet. Such instances were remarkably few in number. When the average breathholding times, expressed in percentage of values obtained after breathing ground air, are plotted against the percentage of change in the pO_2 of the saturated inspired air from ground value, the result is a straight line with a nearly 1:1 relationship when the atmospheric pO_2 is normal (i.e., ground) or less than normal, using air up to 16,000 feet and, using 100 per cent oxygen at altitudes of 35,000 (179 mm.

TABLE I
Breathholding time at different altitudes breathing ambient air

Altitude in feet / Altitude in mm.Hg / pO2 of inspired air	Ground 746 157	7,000 586 123	10,000 523 110	13,000 464 97	16,000 412 87
		Average to nearest second of 2 trials at each altitude			
FE	120	103	93	78	62
E	93	73	64	62	57
FR	97	78	72	63	57
GE	80	67	53	41	39
CL	87	58	61	42	41
SCHU	74	61	58	43	47
RU	125	92	105	78	70
SL	80	61	65	50	41
Mc	67	53	48	52	39
BEC	82	71	66	51	52
SC	72	62	56	75	39
SP	108	87	93	60	49
HU	91	68	60		54
RO	85		59		43
SCH	100		75		58
Breathholding time: average in seconds	91	72	66	57	50
Breathholding time; percentage of ground	100	79	73	63	55
pO2 of inspired air; percentage of ground	100	79	70	63	55
Calculated pO2 of inspired air after saturation with water vapor at 37°C; percentage of ground	100	77	68	60	52

TABLE II
Breathholding time at different altitudes*

Altitude in thousands of feet / Altitude mm. Hg / pO2 of inspired air	Ground 746 157	35 179 179	10 523 110	39 148 148	16 412 87	42.5 125 125	16 O2: 412 412
			Average to nearest second of 2 trials at each altitude				
SC	94	61	75	46	51	33	94
RE	76	70	61	61	48	43	90
BE	66	53	46	42	38	39	90
D	88	78	72	67	53	47	136
P	116	84	74	61	48	48	137
G	96	77	91	61	65	52	162
MO	97	118	75	83	60	43	125
HU	120	98	91	70	60	50	160
BR	105	90		80	55	65	145
RY	61	53	36	47	27	32	84
SCHU	63	65	56	60	50	50	113
MOR	90	73	60	55	43	39	121
SCHI	80	84	58	46	50	44	72
SW	160	118	108	50	65	93	210
BL	167	123	110	73	32	60	210
FL	137	90	90	103	50		135
FI	108	67	72	103	39		125
BEC	106	115	84	75	44		171
Breathholding time; average in seconds	103	88	76	71	58	54	136
Breathholding time; percentage of ground	100	85	74	69	56	52	132
Calculated pO2 of inspired air after saturation with water vapor at 37°C; percentage of ground	100	90	68	69	52	53	248

* At ground, 10,000 feet and 16,000 feet, subjects breathed ambient air. At the higher altitudes, and at 16,000 feet, as indicated in the last column, they breathed 100 per cent oxygen.

TABLE III
Breathholding time breathing different oxygen-nitrogen mixtures by mask

Percentage oxygen content inspired air pO2 of inspired air	100 746	78 582	49 366	35 261	25 187	21* 157	21† 157	10 75
			Average to nearest second of 2 trials with each					
ST	230	209	200	167		116	129	76
WE	142	137	122	114		70	83	43
SC	128	125	114	112		96	97	50
L	226	205	192	169		132	129	75
HU	191	153	158	138		92	102	65
FI	187	163	142	134		102	96	49
FL	210	159	154	123		117	108	58
D	266	240	239	228		154	120	92
BL	186	196	160	152		79	102	34
RE	167	118	122	112		105	105	45
BE	181	125	153	128		81	56	44
SCHN	186	165	157	132		97	80	47
WO	171	136	134	108	114	97	107	41
WI	161	143	124	117	110	99	108	44
Z	151	138	132	103	72	69	93	34
SE	172	171	166	162	157	148	140	58
G	148	137	132	129	116	91	107	33
STO	166	154	137	131	112	81	77	40
STU	183	171	198		130	100	94	52
C	162	148	140		119	113	100	21
K	281	233	219		150	129	125	51
MO	144	146	141	163	124	100	119	55
DR	182	176	142	174	113	91	100	41
Breathholding time; average in seconds	179	163	155	140	120	103	103	50
Breathholding time; percentage of ground	174	158	150	136	117	100	100	49
pO2 of inspired air; percentage of ground	476	373	234	167	119	100	100	48
Calculated pO2 of inspired air after saturation with water vapor at 37°C; percentage of ground	476	372	233	167	119	100	100	48

* From compressed air tank.   † Room air, no mask used.

Hg), 39,000 feet (148 mm. Hg) and 42,500 feet (125 mm. Hg) (Figure 1). However, with pO_2 higher than normal, increasing the pO_2 of inspired air has progressively less effect in increasing the breathholding time; for example, with 100 per cent oxygen (atmospheric pO_2 475 per cent of ground) the breathholding time is increased only to 174 per cent of the ground value. This is illustrated by the loss of the straight line relationship between average breathholding times (expressed as percentage of values of ground air) and percentage of change in pO_2 of saturated inspired air, when the latter is above normal (Figure 2).

DISCUSSION

The data indicate that the effect of the pO_2 of inspired air on the maximum time of breathholding is most striking. Although there are some discrepancies in this relation in certain individuals, it is quite evident that, in this small group, a signifi-

cant decrease in breathholding time as compared to that on the ground can be detected at altitudes as low as 7000 feet. In view of the simplicity of this test, and the short time required to indoctrinate subjects for it and to carry it out, it appears to have value as a method for demonstrating physiological alterations to relatively small changes in the pO_2 of inspired air. Its usefulness in the indoctrination of aviation candidates has already been pointed out (2).

Of more interest is the relation of the change in breathholding time to change in pO_2 at levels less than ground. Here, for every increment of pO_2 change, there is an equal change of increment in the breathholding time. In the case of inspired pO_2 greater than ground values, the effect of the increased tension in lengthening the breathholding time becomes less and less as the pO_2 increases.

This would indicate that at pO_2 of ground level or less, the pO_2 of the inspired air appears to be of
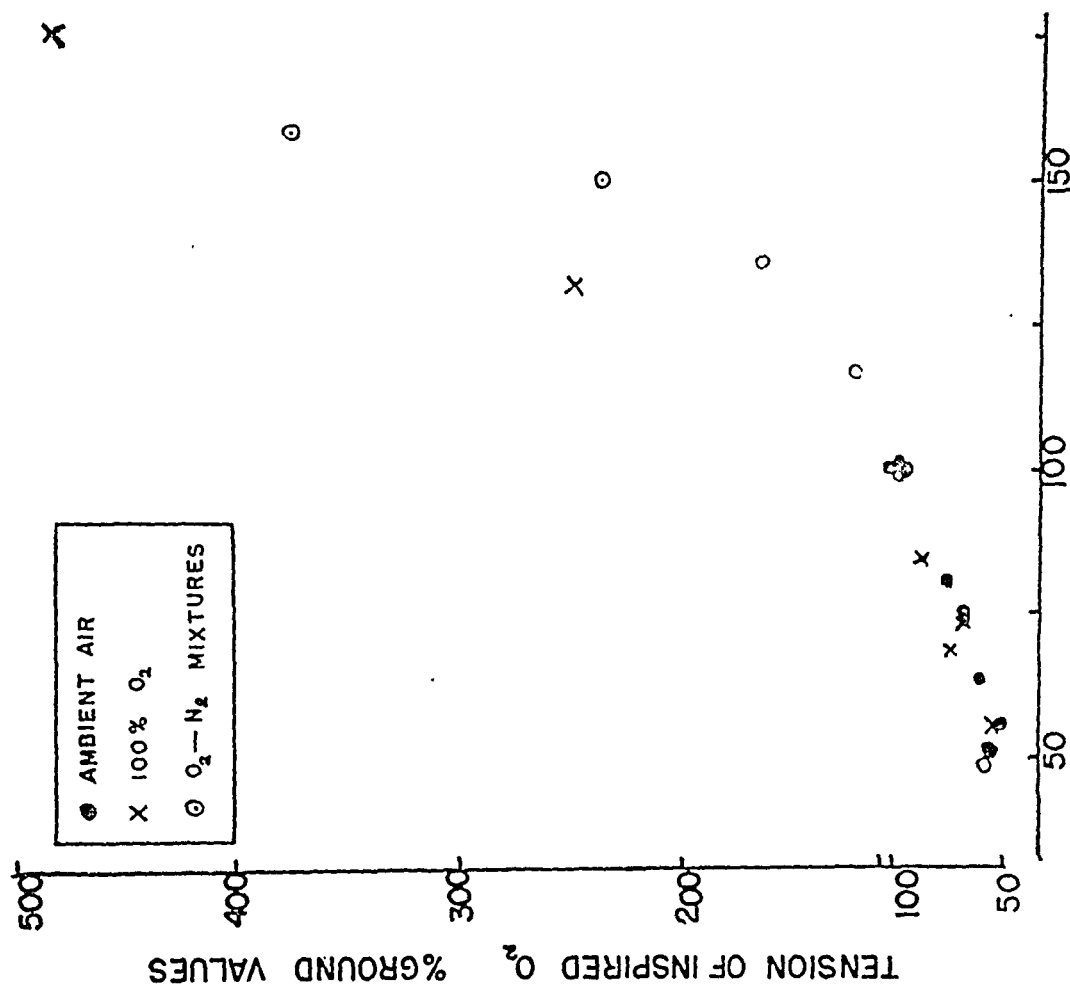
FIG. 2. THE RELATION OF THE pO₂ OF INSPIRED AIR TO THE MEAN BREATHHOLDING TIMES OF THE SUBJECTS IN PERCENTAGE OF "GROUND VALUES"

The pO₂'s range from 75 to 746 mm. Hg.
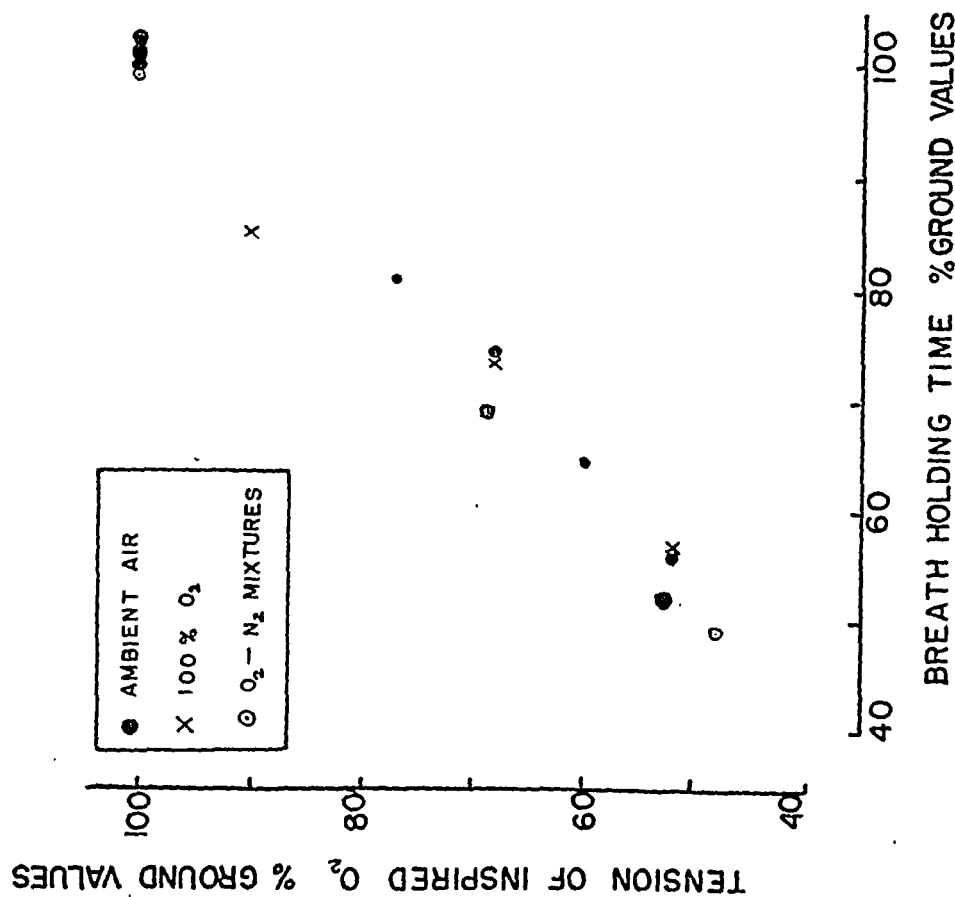
FIG. 1. THE RELATION OF THE pO₂ OF INSPIRED AIR TO THE MEAN BREATHHOLDING TIMES OF THE SUBJECTS, IN PERCENTAGE OF "GROUND" VALUES

All conditions in which the pO₂ of inspired air was normal, or below normal are included.

major importance in determining when the subject shall breathe after maximum breathholding. At pO_2 greater than that on the ground (160 mm. Hg), oxygen, although still influencing the stimulus to breathe, becomes less effective as its tension rises, while another factor (presumably pCO_2 of the blood) becomes more effective as a respiratory stimulant.

It is evident, then, that oxygen lack takes on considerable importance as a respiratory stimulant under certain conditions, and that the relative roles of oxygen and CO_2 in influencing respiration are variable with respect to the oxygen tension of the inspired air.

SUMMARY

From ground levels to altitudes up to 16,000 feet the maximum breathholding time varies in direct proportion to the change in atmospheric pressure (pO_2 of inspired air). Identical changes are noted on the ground when equivalent gas mixtures are inspired by mask. Breathing of oxygen mixtures from 21 to 100 per cent progressively increases the breathholding time, but the effect becomes less and less as the pO_2 approaches 760 mm. Hg.

The breathholding technic offers a simple method for objective demonstration of physiologic changes at relatively low altitudes. A decrease in breathholding time occurred at 7000 feet in all individuals tested.

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VOLUNTARY BREATHHOLDING. III. THE RELATION OF THE MAXIMUM TIME OF BREATHHOLDING TO THE OXYGEN AND CARBON DIOXIDE TENSIONS OF ARTERIAL BLOOD, WITH A NOTE ON ITS CLINICAL AND PHYSIOLOGICAL SIGNIFICANCE [1]

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By means of underwater weighing during breathholding, after preliminary inhalation of gas mixtures containing varying concentrations of oxygen, it has been shown that the diffusion of oxygen from the lungs is influenced by the pO_2 of the inspired air (1). It was also shown (2) that the duration of the maximum voluntary breathholding time was related to the pO_2 of inspired air. Others have shown that alveolar pCO_2 plays an important role in altering the breathholding time (3 to 5). Thus, it is evident that both pO_2 and pCO_2 are factors which influence the time that the breath can be held. Several investigators (6, 3) have studied alveolar gases during breathholding, and at the end of the apneic period produced by hyperventilation, but to our knowledge, the changes in the arterial blood have not been investigated. Despite the lack of knowledge concerning the physiologic changes which occur during breathholding, it is being used with increasing frequency as a clinical test (7 to 9).

In order to clarify the influence of oxygen and CO_2 in regulating the breathholding time, arterial blood gases have been studied during breathholding and at the time when breathing is forced. These data throw considerable light on the interrelation of oxygen and CO_2 as factors which influence respiration under the conditions studied, and on the factors which influence pulmonary diffusion of oxygen and CO_2 during breathholding.

METHODS

Since it was known that the pO_2 of the gas breathed just before breathholding markedly influence the duration of breathholding and the rate of pulmonary diffusion of oxygen, 4 standard conditions were used for study: *1.* Breathholding at ground level after breathing (*a*) ambient air, or (*b*) commercial oxygen (approximately 100 per cent); *2.* Breathholding at 16,000 feet (412 mm. Hg) after breathing (*a*) ambient air, or (*b*) commercial oxygen. Oxygen was given by mask with constant flow.

The subjects were medical students and physicians. All subjects were supine, and the breathholding procedure was initiated after approximately 15 minutes' rest during exposure to the desired pO_2 to be tested. The experiments were conducted in the morning, but without reference to meals. Puncture of the femoral artery was made with an 18-gauge needle which remained in place, obturated, throughout the experiment.

After a time interval sufficient for the subject to relax from the rigors of the arterial puncture, a control arterial sample was collected. The subject then exhaled maximally, inhaled maximally, and held his breath as long as he could. Consecutive arterial samples were taken throughout the breathholding period in some instances, and in others, a sample was collected just before the end of the period. It was not possible to collect samples at the instant that breathholding terminated, but by having the subject signal, samples were collected as close to the breaking time as possible. Samples of 10 to 15 ml. of blood were taken in syringes under 3 to 5 ml. of mineral oil, and immediately transferred to mercury storage vessels containing 0.1 ml. of 30 per cent potassium oxalate, and were stored in a refrigerator until analyzed. One ml. samples were analyzed in duplicate for oxygen and CO_2 content by 2 different analysts, using the Van Slyke manometric apparatus by the method of Van Slyke and Neill (10). Determinations of pH at 36 to 38° C. were made with a MacInnes glass electrode, and Leeds and Northrup potentiometer, using 0.05 molar U. S. Bureau of Standards potassium acid phthalate as reference standard (pH. 4.03 at 38° C.). Wintrobe hematocrit tubes were centrifuged 1 hour at 2500 r.p.m. to determine the percentage by volume of red cells in the blood.

The pCO_2 values were calculated using the nomogram of Hastings and Shock (11) except when the percentage of saturation of the hemoglobin with oxygen dropped below 90 per cent. In such cases, the method of calculation of Van Slyke and Sendroy (12) was employed. The percentage of saturation of the hemoglobin and the pO_2 values were calculated, using the oxygen solubility factors of Sendroy *et al.* (13) and the dissociation curves of normal human blood (14). No corrections for the oxalate were made.

RESULTS

1. Breathholding after inhalation of room air (pO_2, 154 mm. Hg). Seven subjects.

The results are shown in Table I and Figure 1. The marked ventilatory effect of the initial deep breath is indicated by the initial fall in CO_2 and rise in pH (cases 3, 5 and 7). Between 40 and 60 seconds was required for the CO_2 to rise above control values. After this, there was a steady rise in CO_2 and fall in pH, until breathholding was terminated. The oxygen content of arterial blood fell only slightly, except in those subjects who held the breath for relatively long periods of time. The minimum oxygen saturation was 85 per cent after 135 seconds of breathholding. The ventila-
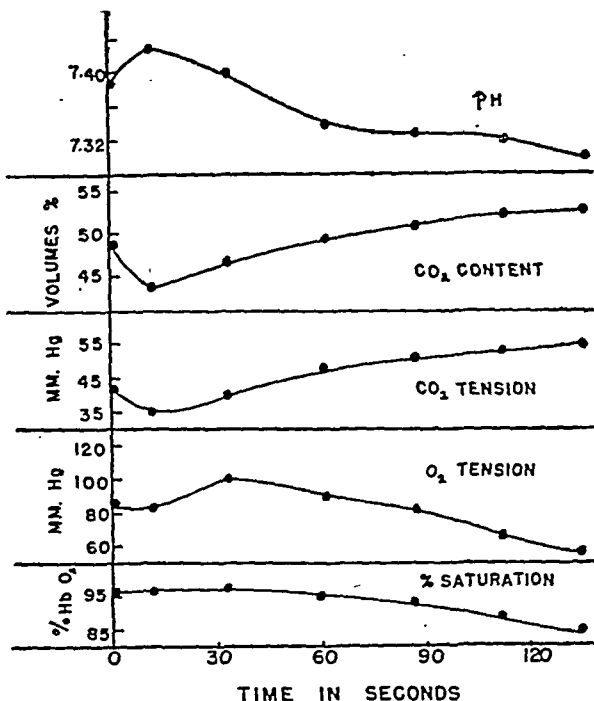
FIG. 1. SERIAL CHANGES IN ARTERIAL BLOOD GASES AND pH DURING MAXIMUM VOLUNTARY BREATHHOLDING FOLLOWING PRELIMINARY BREATHING OF ROOM AIR (pO_2, 154 MM. HG)

TABLE I

Arterial blood gases in breathholding after initial inhalation of room air
($pO_2 = 154$ mm. Hg)

Subject number	Mean time from onset of breath-holding	Sampling time	Maximum breath-holding time	CO₂ content	pH	Oxygen content	Oxygen saturation of hemoglobin	pO₂ (calculated)	pCO₂ (calculated)
	seconds	*seconds*	*seconds*	*vol. per cent*		*vol. per cent*	*per cent*	*mm. Hg*	*mm. Hg*
1	Control			44.8	7.41	20.0	94	77	39
	103	25	120	47.9	7.35	19.4	91	70	48
2	Control			46.1	7.33	20.1	98	123	46
	120	10	120	49.6	7.32	19.0	93	77	51
3	Control			45.0	7.33	19.6	93	79	51
	13	19		39.8	7.44	19.8	94	81	35
	63	12	75	46.5	7.32	19.6	93	79	54
4	Control			46.3	7.39	19.9	98	115	41
	103	15	110	50.3	7.32	19.3	96	70	52
5	Control			49.1	7.38	19.2	100	82	43
	21	9		42.1	7.47	18.7	98	64	32
	35	12		47.3	7.40	19.1	100	80	40
	57	7		49.1	7.40	18.9	99	74	41
	81	9		50.0	7.35	18.8	98	76	48
	109	8	105	50.6	7.35	18.6	97	74	47
6	Control			49.7	7.37	18.6	98	115	46
	145	10	155	53.9	7.31	17.0	90	66	57
7	Control			48.5	7.39	17.3	96	86	42
	11	12		44.1	7.43	17.2	96	84	35
	33	14		47.0	7.40	17.5	97	100	40
	61	15		49.5	7.34	17.2	95	90	48
	87	19		51.0	7.33	16.9	94	82	51
	112	16		52.1	7.32	16.1	89	67	53
	135	11	141	52.6	7.30	15.4	88	58	58

TABLE II

Arterial blood gases in breathholding after initial inhalation of ambient air at 16,000 feet
($pO_2 = 85$ mm. Hg)

Subject number	Mean time from onset of breath-holding	Sampling time	Maximum breath-holding time	CO₂ content	pH	Oxygen content	Oxygen saturation of hemoglobin	pO₂ (calculated)	pCO₂ (calculated)
	seconds	*seconds*	*seconds*	*vol. per cent*		*vol. per cent*	*per cent*	*mm. Hg*	*mm. Hg*
1	Control			43.6	7.41	16.4	78	43	38
	79	22	91	46.2	7.40	14.0	67	34	41
2	Control			48.1	7.41	15.2	75	41	41
	83	10	88	49.6	7.40	14.5	72	38	43
3	Control			46.1	7.42	13.9	66	34	43
	12	16		40.7	7.48	17.6	84	46	33
	56	12	62	46.1	7.40	13.6	65	34	44
5	Control			45.1	7.48	16.0	84	40	33
	59	8	65	47.3	7.44	14.1	74	34	36
6	Control			48.8	7.42	14.0	74	40	40
	12	10		45.4	7.49	17.0	88	50	32
	37	14		49.8	7.41	14.5	77	43	42
	63	18		51.0	7.41	12.6	67	34	43
	91	18		51.5	7.41	10.8	57	29	44
	122	24	140	53.3	7.40	8.4	44	24	46
7	Control								
	16	8		47.6	7.42	12.0	67	34	38
	84	13	90	51.1	7.39	8.3	46	25	42
8	Control			48.5	7.41	15.0	70	35	46
	67	10	72	49.9	7.39	13.9	65	33	49
9	Control			45.6	7.43	15.9	83	49	41
	65	16	75	49.4	7.41	14.4	75	41	47
10	Control			47.8	7.43	14.2	80	44	41
	60	10	68	49.8	7.43	12.0	68	34	44

tory effect of the initial deep breath is barely evident in so far as oxygen content is concerned, presumably because the resulting increase in pO_2 has little effect in increasing the oxygen content.

2. Breathholding after inhalation of ambient air at 16,000 feet (pO_2, 85 mm. Hg). Ten subjects.

The results are shown in Table II and Figure 2. The maximum breathholding time of each subject is definitely shorter than that after preliminary inhalation of ambient air. In 7 subjects who had both tests, the average breathholding time after inhalation of room air was 120 seconds, and after inhalation of ambient air at 16,000 feet, was 90 seconds. The ventilatory effect of the initial deep breath is now evident not only with respect to arterial CO_2 and pH, but also with respect to oxygen content. The control values for arterial oxygen saturation averaged 80.5 per cent

at this altitude, so that changes in pO_2 resulting from the deep breath produced larger changes in the oxygen content of arterial blood. The total change in arterial CO_2 and pH was less than after inhalation of room air, because of the shortened breathholding time, but the rate of rise in CO_2 content was essentially the same. The oxygen content, however, fell consistently at a much faster rate than after initial inhalation of room air, and reached levels as low as 46 per cent saturation in 84 seconds.

3. Breathholding after inhalation of 100 per cent oxygen at an altitude of 16,000 feet (pO_2, 410 mm. Hg). Four subjects.

The results are shown in Table III. The rate of change in CO_2 content and pH appears to be similar to the above categories. However, higher levels of CO_2 and lower levels of pH were reached because of the longer periods of breathholding.

TABLE III

Arterial blood gases in breathholding after initial inhalation of 100 per cent oxygen at 16,000 feet
(pO_2 = 410 mm. Hg)

Subject number	Mean time from onset of breath-holding	Sampling time	Maximum breath-holding time	CO_2 content	pH	Oxygen content	Oxygen saturation of hemo-globin	pO_2 (calculated)	pCO_2 (calculated)
	seconds	*seconds*	*seconds*	*vol. per cent*		*vol. per cent*	*per cent*	*mm. Hg*	*mm. Hg*
3	Control			45.5	7.31	21.1	100	130	54
	12	20		40.7	7.42	20.9	100	120	38
	67	21	100	47.0	7.31	21.0	100	130	56
8	Control			44.8	7.42	21.8	100	121	42
	118	19	130	50.9	7.32	22.2	100	260	60
9	Control			41.7	7.50	21.1	100		32
	205	16	215	53.0	7.31	21.0	100		64

Despite the relatively long periods of breathholding, there was no measurable change in the arterial oxygen content after periods of breathholding up to 205 seconds.

4. Breathholding after inhalation of 100 per cent oxygen at ground level (pO_2, 740 mm. Hg). Four subjects.

The results are shown in Table IV, Figure 3. As compared to breathholding after inhaling room
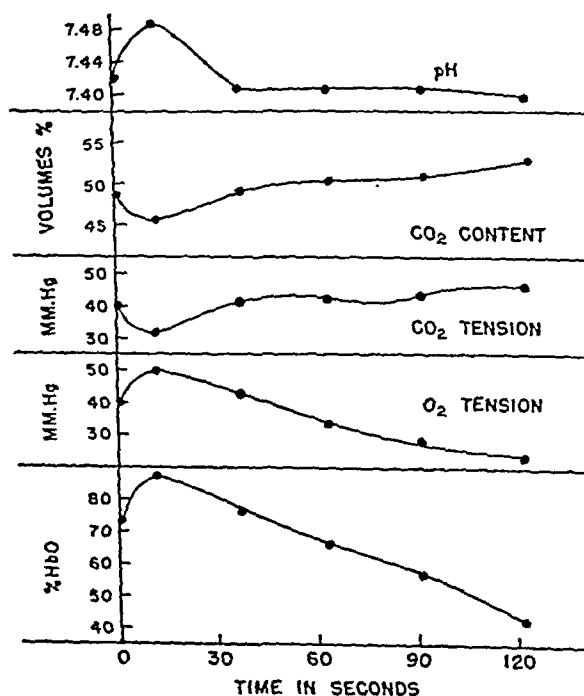
FIG. 2. ARTERIAL BLOOD CHANGES DURING BREATH-HOLDING AFTER INHALATION OF AMBIENT AIR AT 16,000 FEET (pO_2, 85 mm. Hg)
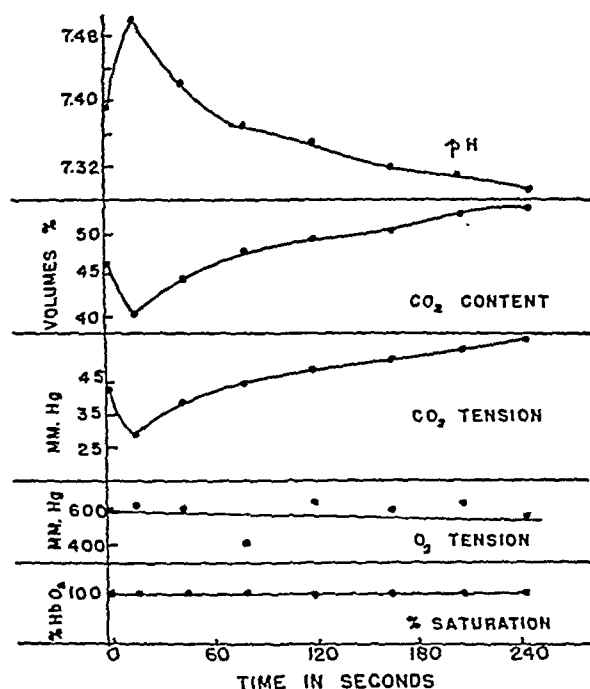
FIG. 3. ARTERIAL BLOOD CHANGES DURING BREATH-HOLDING AFTER PRELIMINARY INHALATION OF 100 PER CENT OXYGEN (pO_2, 740 mm. Hg)

air, the breathholding time was prolonged in each subject (average of 114 seconds after inhalation of room air, and of 214.5 seconds after inhalation of 100 per cent oxygen on the ground). The rate of change in CO_2 content and pH were again similar to the other categories; however, the oxygen content and saturation remained unchanged after periods of breathholding as long as 241 seconds.

The above results may be summarized as showing that during breathholding after inhalation of

TABLE IV

Arterial blood gases in breathholding after initial inhalation of 100 per cent oxygen at ground level
($pO_2 = 740$ mm. Hg)

Subject number	Mean time from onset of breath-holding	Sampling time	Maximum breath-holding time	CO₂ content	pH	Oxygen content	Oxygen saturation of hemo-globin	pO₂ (calculated)	pCO₂ (calculated)
	seconds	*seconds*	*seconds*	*vol. per cent*		*vol. per cent*	*per cent*	*mm. Hg*	*mm. Hg*
1	Control								
	213	25	227	52.3	7.25	22.1	100	440	63
2	Control			46.4	7.39	21.5	100	430	41
	200	10	205	51.6	7.29	22.0	100	310	55
4	Control			46.7	7.37	21.8	100	610	43
	18	15		40.3	7.50	21.9	100	650	29
	44	18		45.4	7.42	21.8	100	630	38
	80	25		48.0	7.37	22.2	100	410	45
	121	22		49.7	7.35	21.9	100	660	49
	163	35		50.1	7.32	21.8	100	620	51
	207	27		52.1	7.31	21.9	100	650	55
	241	22	256	52.4	7.29	21.7	100	580	58
5	Control			44.6	7.45	20.6	100	257	35
	161	12	170	51.5	7.31	20.8	100	330	53

varying pO_2, the arterial CO_2 content and pH rise steadily, and that the final value reached depends upon the duration of breathholding. The arterial oxygen content falls rapidly to very low levels during breathholding after inhalation of 10 per cent oxygen, falls less rapidly after inhaling 21 per cent oxygen, and falls little, if any, after inhalation of supra-normal mixtures, despite the fact that the breath is held for progressively longer periods of time.

FIG. 4. THE INTERRELATION OF ARTERIAL BLOOD pO₂ AND pCO₂ AT THE TERMINATION OF MAXIMUM VOLUNTARY BREATHHOLDING, AFTER PRELIMINARY BREATHING OF VARIABLE TENSIONS OF OXYGEN

Each type of symbol represents an individual subject.

The arterial blood gas levels at the end of voluntary breathholding are consistent with the alveolar gas tensions which Douglas and Haldane (6) obtained at the end of the apneic period induced by hyperventilation. They noted that at alveolar pO_2 values above 120 mm. Hg, the apnea terminated when the pCO_2 reached 45 to 50 mm. Hg, while at pO_2 levels below 120 mm. Hg, the pCO_2 at the end of apnea varied with the pO_2.

DISCUSSION

It has been shown that during breathholding the rate of exchange of gas between the lungs and the blood is relatively constant. When the arterial gas findings during breathholding after inhalation of varying pO_2 are considered together, it is clear that the oxygen is falling, and the CO_2 and pH are rising, the former at a rate dependent chiefly upon the pO_2 of inspired air and the oxygen dissociation curve of arterial blood, and the latter 2 at a more constant rate, dependent chiefly on CO_2 production and the CO_2 dissociation curve, once the transient effect of the initial deep breath is over. Since the breathholding time (breaking point) varies with the pO_2 of inspired air (2), we are, in effect, studying the interrelation of arterial oxygen and CO_2 as respiratory stimulants with both variables changing: CO_2 at a relatively constant rate under all conditions studied, and oxygen at a variable rate, dependent upon

FIG. 5. THE INTERRELATION OF ARTERIAL BLOOD OXYGEN CONTENT AND pCO_2 AT THE TERMINATION OF MAXIMUM VOLUNTARY BREATHHOLDING

The lines joining the symbols represent individual subjects numbered according to the tables.

both the pO_2 of inspired air and the arterial oxygen dissociation curve.

When the arterial pCO_2 and pO_2 at the breaking point are related as factors in inducing breathing (Figure 4), there appears to be some interrelation at arterial pO_2 below 100 mm. Hg, but very little at higher pO_2. However, when the arterial pCO_2 is plotted against oxygen *content* of arterial blood, instead of pO_2 (Figure 5), a series of parallel lines are obtained, which indicate a fairly constant interrelation of pCO_2 and *oxygen content* to the breaking point, regardless of the pO_2 of initially inspired air. Inspection of the curve denoting the relationship of the pO_2 of initially inspired air to the breathholding time (2, Figure 2) shows that its shape is roughly like that seen in Figure 4. If the values for pO_2 of inspired air (2, Figure 2) are calculated in terms of the approximate oxygen content of whole blood to be expected, and this plotted against breathholding time, again the relationship of oxygen content of whole blood to the breathholding time becomes a relatively constant one at all pO_2's breathed (Figure 6).

By utilizing varying amounts of hyperventilation, Mirsky and Grinker (15) have recently tested the effect of variable initial pCO_2 at relatively constant initial pO_2 of inspired air, on the breathholding time, and have found that the breathholding time varies with the amount of hyperventilation. Others (3 to 6) have also demonstrated the effect of CO_2 on the breathholding time; in fact, hyperventilation is an age-old method for lengthening the breathholding time.

It would appear, then, that in so far as breathholding time is concerned, arterial oxygen and CO_2 are interrelated as factors which initiate breathing, regardless of the range of the pO_2 and pCO_2 of the inspired air and blood, but that the influence of pO_2 becomes less and less as it rises above normal (about 100 mm. Hg). The interrelation becomes a reasonably constant one if the oxygen is considered in terms of oxygen content of whole arterial blood, and hence, of the ability of the blood to deliver a given volume of oxygen to the tissues in order to maintain an adequate pO_2 there. Such a relation is quite consistent with the concept that the stimulus to breathe is dependent on the metabolic state of the cells of the respiratory centers, and that pO_2 and pCO_2 in the blood influence respiration to the extent that they influence the metabolic requirements of the centers. Higher pO_2 of inspired air and blood become less and less effective in increasing the breathholding time, because once the hemoglobin is saturated, additional oxygen can be delivered to the tissues only to the extent that it dissolves in blood plasma.

THE CLINICAL AND PHYSIOLOGICAL SIGNIFICANCE OF BREATHHOLDING

Since the observations presented in the 3 papers of this series include related phases of the phenomenon of breathholding, it is perhaps well to discuss the clinical and physiological significance of these findings together. Of particular significance are those findings related to diffusion of gas through the lungs, and those concerned with the interrelation of oxygen and CO_2 in stimulating the taking of a breath.

During breathholding over the periods of time utilized in these experiments, it is evident that the bellows effect of breathing is eliminated, and the passage of oxygen and CO_2 between the pulmonary dead space and the arterial blood is essentially a phenomenon of diffusion. Since, under normal conditions, the tensions in the alveoli are essentially
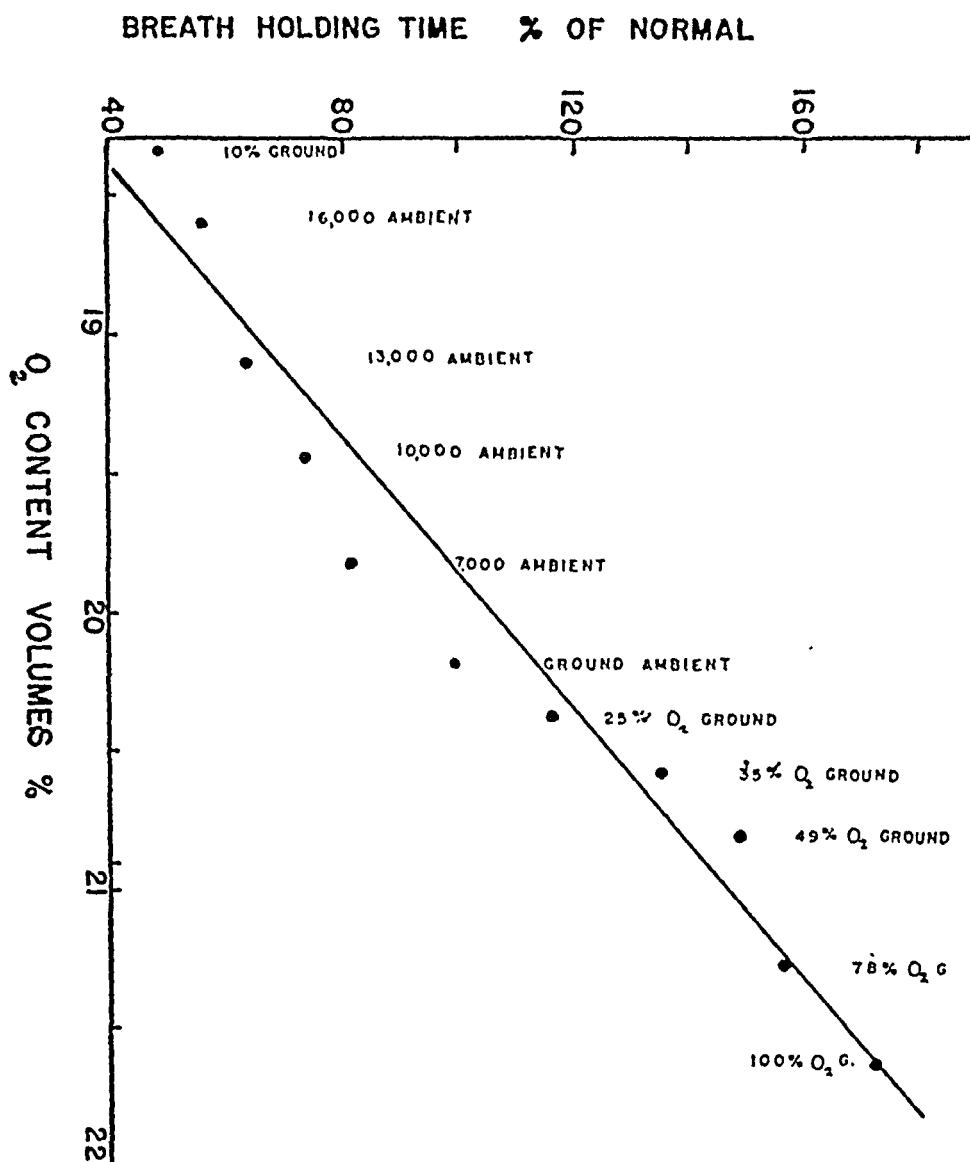
FIG. 6. THE RELATION OF THE BREATHHOLDING TIME TO THE INITIAL
OXYGEN CONTENT OF WHOLE ARTERIAL BLOOD

Calculated from the pO_2 of the inspired air preliminary to breathholding.
The calculations were made from the data in Paper II.

the same as in arterial blood,[2] the pulmonary diffusion gradient can be assumed to lie largely between the pulmonary dead space and the alveoli. Therefore, for purposes of this discussion, pulmonary diffusion will be treated as a phenomenon of gaseous diffusion within the tubular structures of the lung. Since, at most, only a minor portion of the total pulmonary diffusion gradients for CO_2 and oxygen can lie between the alveoli and arterial blood (membrane diffusion), the neglect of this

factor, by assuming arterial and alveolar tensions to be equal, will not alter the basic principles to be evolved from the data at hand.

In analyzing the fate of oxygen and CO_2 in both lungs and arterial blood, and the factors which influence their passage during breathholding, it is necessary to consider whether oxygen consumption and CO_2 production by the tissues is significantly altered under the conditions studied. Variations of pO_2 over the range of these experiments apparently does not influence significantly either oxygen consumption or CO_2 production (16, 17). The similarity in the rates of arterial CO_2 retention at the different pO_2 of inspired air, likewise

[2] The similarity between alveolar and arterial pCO_2 is well established; however, only recently has it been demonstrated that there is no significant physiological difference between alveolar and arterial pO_2 (22).

suggests that variations in oxygen consumption and CO_2 production are not of importance in influencing the results of these experiments.

Likewise, variations in cardiac output, due either to mechanical or chemical factors, can scarcely be of importance in influencing our results. Since the technique of breathholding was similar under all conditions, mechanical effects on cardiac output cannot explain the variations caused by altering the pO_2 of inspired air. Although variations in cardiac output, pulse rate and blood pressure may result from breathholding, these effects are relatively insignificant, except in the so-called group of hyper-reactors (7).

It has generally been assumed that CO_2 diffuses much more rapidly than oxygen through the body. This is undoubtedly true as it applies to diffusion of CO_2 in solution in body fluids (18). However, these studies show conclusively that in so far as gaseous CO_2 is concerned, it diffuses much more slowly through the lungs than does oxygen, not only when the pO_2 of inspired air is normal or supranormal, but even when it is reduced. There are 2 reasons for this. First, CO_2 has a larger molecular weight than oxygen, and hence its coefficient of diffusion is less. ($CO_2 = 0.139$ sq. cm. per second; oxygen $= 0.178$ sq. cm. per second.) Second, when one compares the relative diffusion gradients of CO_2 and oxygen between the pulmonary dead space and the alveoli under the conditions of these experiments, it is evident that the diffusion gradient for CO_2 may be much less than that for oxygen.

The gaseous diffusion gradient in the lungs, P_d, equals $P_1 - P_2$, where $P_1(CO_2)$ is the pCO_2 in the alveolus (or arterial blood), and $P_2(CO_2)$ is the pCO_2 in the pulmonary dead space. $P_1(O_2)$ is the pO_2 in the pulmonary dead space, and $P_2(O_2)$ is the pO_2 in the alveolus (or arterial blood).

At the beginning of breathholding after a deep breath, $P_1(CO_2)$ is about 34 mm. Hg (see charts), and $P_2(CO_2)$ can be assumed to be zero or greater. As the breath is held, $P_1(CO_2)$ gradually rises, but is less than 60 mm. Hg, even after 4 minutes of breathholding. During this period $P_2(CO_2)$ probably rises slowly, but cannot fall; hence, the maximum CO_2 diffusion gradient during breathholding is less than 60 mm. Hg, and is probably of the order of 34 mm. Hg, under all the conditions of these experiments. Since $P_2(CO_2)$

cannot be less than zero, the limitation of the CO_2 diffusion gradient is largely controlled, therefore, by the CO_2 dissociation of blood, which allows the CO_2 being produced to dissolve in the blood with a relatively small increase in $P_1(CO_2)$.

After the initial deep breath $P_1(O_2)$ is about 700 mm. Hg after 100 per cent oxygen inhalation, 150 mm. Hg after inhalation of room air, and 85 mm. Hg after inhalation of air at 16,000 feet. All these pressures are much higher than $P_1(CO_2)$. As for $P_2(O_2)$ it is roughly 400 to 500 mm. Hg after 100 per cent inhalation of oxygen, 100 mm. Hg after inhalation of room air, and 50 mm. Hg after inhalation of air at 16,000 feet. While a decrease in $P_2(CO_2)$ is limited by the fact that it cannot fall below zero, $P_2(O_2)$ can be decreased markedly, depending on the oxygen dissociation curve of whole blood and the oxygen consumption. At normal or higher pO_2 of inspired air $P_2(O_2)$ can fall greatly with relatively slight change in oxygen content. Thus, as compared to CO_2, the initial pulmonary oxygen diffusion gradient is high, and the arterial oxygen dissociation is such that a relatively high pO_2 gradient can readily be maintained during breathholding. Since the rate of change in lung volume during breathholding, which is largely due to oxygen diffusion, is constant (1), it may be assumed that the oxygen diffusion pressure gradient established at the beginning of breathholding remains relatively constant throughout.

Both these diffusion factors, diffusion gradient and diffusion coefficient, tend to cause oxygen to diffuse more rapidly than CO_2 through the lungs, and offer an explanation of why, during breathholding, oxygen diffuses out of the lungs infinitely more rapidly than CO_2 diffuses into the lungs. Moyer and Beecher (19) came to a similar conclusion with respect to inhalation of 100 per cent oxygen in dogs anesthetised with barbiturates, when they found that the CO_2 content of arterial blood rose, while the oxygen saturation remained normal, during depressed breathing. Our observations would suggest that even at normal pO_2 of inspired air (21 per cent oxygen), oxygen can be taken in more effectively than CO_2 can be eliminated, when the need arises. This is consistent with many observations, indicating that, under physiologic conditions, respiratory ventilation is influenced more by changes in pCO_2 of in-

spired air and arterial blood, than by comparable changes in pO_2, for during homeostasis, breathing must be adjusted primarily to equilibrate the least diffusible gas, namely CO_2. It does not necessarily mean, however, that the respiratory centers are more sensitive to changes in CO_2 than to oxygen, as many observers have concluded. As our data indicate, the error in this reasoning lies in the fact that the pCO_2 and pO_2 in the tissues is related to the tension of these gases in the alveoli and blood principally, according to the capacity of the blood to carry these gases in quantities consistent with metabolic demands.

Because of the fact that respiration serves as a homeostatic mechanism, and the bellows effect of breathing adjusts itself to maintain the blood and tissue gas tensions at optimum levels for tissue function, the blood gases do not reflect functional changes in pulmonary exchange until an extreme degree of impairment is present. While such clinical measurements as respiratory rate, tidal air, vital capacity, lung volume, complemental and supplemental air, and respiratory minute volume are undoubtedly of great clinical value in estimating pulmonary function, yet a clear understanding of the significance of these tests in terms of diffusion capacity of the lungs is lacking. Direct measurements of changes in lung volume and/or arterial blood gases during breathholding should lead to a better understanding of the diffusion capacity of the lungs in health and disease.

· The specific effects of oxygen and CO_2 on the breathholding time suggest, also, that the duration of voluntary breathholding may offer a simple means for studying respiratory factors in unanesthetized man. The finding that oxygen and CO_2 are reciprocally interrelated with respect to respiratory activity in an organized fashion over a wide range of pO_2 and pCO_2, tends to clarify a great deal of the controversy concerning the relative effects of oxygen lack and CO_2 as respiratory stimulants. Recent reviews of this subjects have been written by Bernthal (21) and Schmidt (22). Our observations indicate that in so far as respiratory activity after breathholding is concerned, the composite respiratory mechanism has no true threshold for either oxygen or CO_2 nor is there any particular point where oxygen lack or CO_2 alternately take over control of respiration, when considered in the light of the capacity of the blood to carry oxygen and CO_2 to and from the respiratory centers. In so far as the breathholding time is concerned, the effects of oxygen and CO_2 (or pH) on it can best be explained by their alteration of the tonic activity of the respiratory tissues in response to metabolic need.

In utilizing the breathholding time as an index of respiratory activity, it must be recognized that there are many factors which influence it, some related to chemical factors, and some probably not.

For instance, under controlled conditions with respect to preliminary breathing, oxygen content of inspired air, learning, etc. the breathholding time has varied from 56 to 167 seconds among our normal subjects. In some of our subjects, it has varied significantly from time to time. When the breath is held after an initial inspiration, the breathholding time is longer than after an initial expiration. Breathholding time appears to correlate poorly with vital capacity (5) and exercise tolerance tests (20). Schneider (5) feels that psychological factors play an important role in determining the breathholding time. Mirsky and Grinker (15) have compared the breathholding time in normal controls with that of patients suffering from anxiety states. They found that the mean breathholding time was significantly lower in the anxious group than in the normals. Whether these differences in the breathholding time of normal and anxious individuals are related to differences in metabolic requirements in the 2 groups, or to differences in their ability consciously or unconsciously to withstand the increasingly unpleasant sensations incident to breathholding, cannot be established at this time.

The variability of the breathholding time within and among individuals does not preclude it as a useful clinical test, but does indicate the restrictions which must be placed on its use as a means of studying respiratory mechanisms in conscious human subjects, and the need to learn more about the factors which control the breathholding time.

SUMMARY AND CONCLUSIONS

1. Serial measurements of oxygen, CO_2, and pH of arterial blood throughout the period of maximum voluntary breathholding have been made, after preliminary inhalation of oxygen mixtures varying from pO_2 of 85 to 740 mm. Hg.

2. Oxygen and CO_2 are interrelated as factors which influence the breathholding time, regardless of the relative tensions of either of these gases in the blood. The effectiveness of supra-normal pO_2 in lengthening the breathholding time is lessened, not because oxygen loses its action on respiratory tissues, but because once the hemoglobin is saturated, the blood is less effective in delivering additional oxygen to the tissues.

3. The observations presented in these 3 papers suggest that breathholding technics may be applied to the study of pulmonary diffusion, and of factors related to respiratory control in man.

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FLUID LOSS IN RATS WITH TOURNIQUET SHOCK [1]

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Release of a high unilateral tourniquet producing complete interruption of blood flow to a hind limb of the rat for about 5 hours almost invariably results in fatal shock. The development of shock is accompanied by progressive edema of the limb. The main object of the following experiments was to determine the magnitude and the rapidity of the fluid loss in the injured extremity.

METHODS

Adult white male rats weighing about 150 to 300 grams were used. These were anesthetized with sodium pentobarbital, given intraperitoneally in dosage of 25 to 35 mgm. per kgm. of body weight (0.3 to 0.4 ml. of a 2 per cent solution). Circulation in the left lower extremity was then entirely occluded by 2 tightly wound rubber band tourniquets. The first was placed around the knee in order to obtain extension of the leg. This facilitated the application of the second tourniquet, which was placed as high as possible in the groin, anchorage being obtained over a redundant segment of skin drawn down beneath the rubber band.

Constriction was maintained for 5 hours and 10 minutes, at the end of which time the rats were alert and showed complete outward recovery from the anesthesia. The animals were then sacrificed by decapitation, either without release of tourniquet, or at fixed time intervals after release, i.e., 15 minutes, 30 minutes, and 1, 2 and 3 hours. The experiments were performed with small groups of rats sacrificed at different periods by random selection, until there was a total of 20 rats for each time interval.

In each group there were 1 or more control rats which developed shock after removal of tourniquet and went on to death. Since these yielded only little blood at the time of death, an additional group of 11 rats was sacrificed at 5 to 6 hours following removal of constriction, in order to obtain sufficient blood for chemical determinations. In 18 rats sacrificed by ether at various intervals after tourniquet release, the figures for fluid loss were of the same order as those obtained from decapitated animals.

There were a few deaths apparently due to anesthesia. In addition, several rats which chewed their leg before or after removal of tourniquet were discarded. Food and water were withheld for a period of about 10 hours be-

fore anesthesia was given, and also during the course of the experiments. The latter were performed at room temperature during the summer and autumn seasons.

The amount of fluid lost in the leg following removal of tourniquet was determined by the method of bisection (1, 2). An anterior midline incision was made from sternum to symphysis pubis and followed by evisceration. A similar posterior midline incision extended down across the base of the tail. The spine was then transected at about the level of the first or second lumbar vertebra, and the lower extremities separated by bilateral paravertebral incisions passing through the hip joints.

The difference in weight between the normal and the edematous extremity gave the gain in weight of the latter due to accumulation of fluid. For convenience, this increase was expressed in terms of percentage of total body weight.

In addition to local fluid loss, other items studied were: (1) hemoconcentration, (2) total protein of edema fluid and blood serum, and (3) blood serum creatine.

Hemoconcentration was measured by hematocrit (3) with powdered heparin as anticoagulant. Control values were obtained on all rats prior to application of tourniquet, and again at the time of sacrifice. This afforded a comparison between progressive loss of fluid and hemoconcentration.

Total protein was determined by the falling drop method (4). Edema fluid was secured from the legs of rats sacrificed at 2, 3 and 5 to 6 hour intervals following release of the tourniquet. Incisions of skin, subcutaneous tissue and fascial planes were made, and pale pink fluid obtained on the surface of a scalpel blade by gentle pressure. This was transferred to a small dish containing powdered heparin to prevent clotting. Incision of muscle was avoided in order to prevent contamination with blood.

Creatine was determined as follows: a Folin-Wu filtrate was acidified to a final concentration of 1 N HCl, and then autoclaved at 15 pounds pressure for 20 minutes. After cooling, an amount of NaOH equivalent to the HCl was added. The remainder of the procedure was the same as for creatinine (5), the creatine level being obtained by the difference in the 2 values.

Autopsies were performed on most rats, and sections obtained from various organs for microscopic study.

RESULTS

Control rats. There were 15 control rats, all of which died in shock following removal of the tourniquet. The period of survival ranged from 2 to

23 hours, with an average of 7½ hours. Bisection of 9 animals gave a mean increase in weight of the injured leg equal to 4.8 per cent of body weight, and a range of 4.3 to 5.7 per cent. Hemoconcentration was marked at the time of death, the mean levels of the normal and experimental hematocrits being 48.7 and 77.8 respectively. The normal hematocrits ranged from 46.0 to 52.5, and the experimental from 72.5 to 82.5.

TABLE I

Fluid loss

| Time of sacrifice after release of tourniquet* | Percentage of body weight | |
	Mean	Range
None†	1.1	0.8 to 1.4
15 minutes	2.1	1.6 to 2.6
30 minutes	2.8	2.3 to 3.5
1 hour	3.4	3.0 to 4.1
2 hours	4.2	3.4 to 4.7
3 hours	4.8	4.0 to 6.2

* 20 rats were sacrificed at each time interval.
† Rats sacrificed after 5 hours and 10 minutes without release of tourniquet.

Local fluid loss. Table I shows the amount of fluid, expressed as percentage of total body weight, lost into the extremity before and at various time intervals after release of tourniquet. The rate of extravasation is indicated by the graph in Figure 1.

In rats sacrificed after a 5-hour and 10-minute

FIG. 1. MEAN FLUID LOSS

period of constriction with the rubber bands still in place, the extremity showed a mean increase in weight equal to 1.1 per cent of body weight. Presumably this was due to blood trapped in the leg by the tourniquet, and also to the development of edema proximal to the rubber band. Fifteen minutes after removal of the tourniquet, the mean fluid loss amounted to 2.1 per cent of body weight, rose to 2.8 per cent at 30 minutes, and then to 3.4, 4.2 and 4.8 per cent at 1, 2 and 3 hours respectively. Thus, there was rapid loss of fluid in the first 15 minutes following release of tourniquet, a slightly reduced but still rapid extravasation during the next 15 minutes, and thereafter a distinct and progressive diminution in the rate of loss. The increments in leg weight per 15 minutes following tourniquet removal were 1 per cent of body weight in the first 15 minutes, 0.7 per cent in the following 15 minutes, 0.3 per cent in the next ½ hour, 0.2 per cent during the second hour, and 0.15 per cent during the third hour.

TABLE II

Hemoconcentration

| Time of sacrifice after release of tourniquet | Normal hematocrit | | Experimental hematocrit | | Increase in hematocrit |
	Mean	Range	Mean	Range	
None	47.8	42 to 52	49.0	46 to 57	1.2
15 minutes	46.9	43 to 50	56.6	54 to 60	9.7
30 minutes	46.7	44 to 50	58.6	53 to 66	11.9
1 hour	47.5	43 to 51	61.7	59 to 68	14.2
2 hours	46.6	36 to 51	65.1	58 to 71	18.5
3 hours	47.9	45 to 51	71.3	62 to 83	23.4
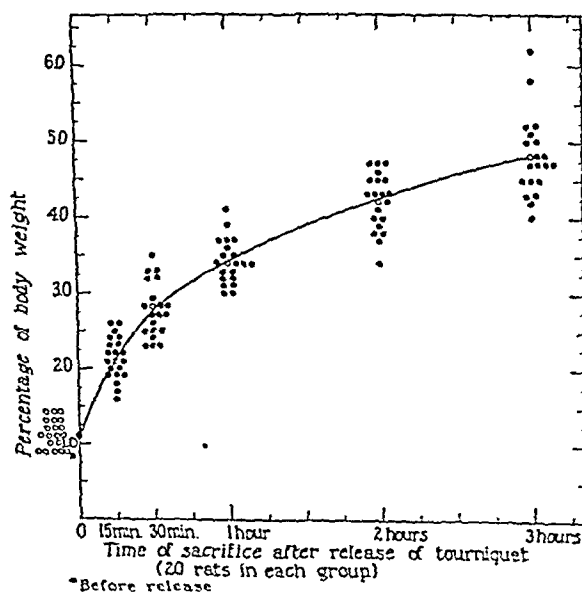
Hemoconcentration. The mean values and the range of experimental and normal hematocrits are given in Table II. In rats sacrificed after 5 hours and 10 minutes of constriction without removal of the tourniquet, the change in hematocrit was variable, *i.e.*, either a slight rise or fall, or practically no change. Although bisection of the 20 rats sacrificed at this time revealed an average increase in weight of the injured leg equal to 1.1 per cent of body weight, there was an average increase of only 1.2 in hematocrit. After removal of the tourniquet the hematocrit rose sharply, and at the end of 15 minutes there was a mean increase of 9.7 over the normal. At the end of 30 minutes the mean increase was 11.9, and thereafter the values rose to 14.2, 18.5, and 23.4 at 1, 2 and 3 hours respectively.

The greatest increment in hematocrit occurred in the first ¼ hour after removal of tourniquet, corresponding to the period of most rapid fluid loss into the extremity. The increment was considerably reduced during the next 15 minutes, even though local fluid loss was still rapid. In the following 2½ hours, as fluid loss diminished progressively, the hematocrit continued to rise slowly, and after the first hour at the approximate rate of 1 unit per 15 minutes.

Total protein of edema fluid and blood serum. The protein content of the edema fluid in the injured leg was determined in 37 rats. This fluid was obtained from animals sacrificed at 2, 3 and 5 to 6 hours after release of tourniquet, and the mean values at these periods were 6.1, 5.7, and 5.4 grams per 100 ml. respectively. Such levels amounted to approximately 68 to 80 per cent of the corresponding values for serum protein (Table III). The figures for the latter did not differ

TABLE III
Total protein of edema fluid

Time of sacrifice after release of tourniquet	Number of rats with determinations	Grams per 100 ml.		Corresponding serum protein	
		Mean	Range	Mean	Range
2 hours	8	6.1	5.2 to 6.9	7.6	7.2 to 7.8
3 hours	18	5.7	4.7 to 6.5	7.1	6.7 to 7.8
5 to 6 hours	11	5.4	4.9 to 6.5	8.0	7.6 to 8.3

appreciably at any of the time intervals after tourniquet removal, and were also within the range of determinations on 10 normal rats which averaged 7.4 grams, and varied from 7.0 to 7.8 grams (Table IV). Thus progressive fluid loss and hemoconcentration were not accompanied by a significant rise or fall in serum protein.

TABLE IV
Total serum protein

Time of sacrifice after release of tourniquet	Number of rats with determinations	Grams per 100 ml.	
		Mean	Range
None	19	7.8	5.9 to 9.3
15 minutes	19	8.0	6.2 to 10.2
30 minutes	13	7.9	6.6 to 10.0
1 hour	13	8.0	6.4 to 11.4
2 hours	8	7.3	5.9 to 8.4
3 hours	5	7.1	6.7 to 7.8

Serum creatine. Determinations of serum creatine in 10 normal rats gave a mean level of 12.9 mgm. per 100 ml., and a range of 9.0 to 15.9. In rats sacrificed after 5 hours and 10 minutes of constriction of a hind limb with tourniquet still in place, there was no significant change in this value, i.e., in 19 rats the mean level was 10.9 mgm., and the range 8.7 to 13.1. However, following release of the tourniquet there was a rapid and steady rise in serum creatine (Table V). This amounted to

TABLE V
Serum creatine

Time of sacrifice after release of tourniquet	Number of determinations	Mgm. per 100 ml.	
		Mean	Range
None	19	10.9	8.7 to 13.1
15 minutes	19	20.1	16.7 to 24.7
30 minutes	13	27.4	15.9 to 34.3
1 hour	13	29.4	23.6 to 38.5
2 hours	7*	35.1	24.4 to 40.0
3 hours	5*	40.2	37.6 to 43.8
5 to 6 hours	3*	64.5	59.2 to 68.9

* Determinations made on pooled blood, usually from 2 or 3 rats.

an increase over the normal of approximately 100 per cent at the end of ½ hour, 200 per cent at the end of 3 hours, and 400 per cent between 5 and 6 hours. Again, the increment was largest in the first 2 fifteen-minute periods following removal of the tourniquet, after which a progressive rise was maintained at a slower rate during the next 5 hours.

Serum creatinine was slightly elevated during the course of the experiments, probably as a result of renal failure. From a mean level on 10 normal rats of 1.4 mgm. per 100 ml., the mean values rose to 2.8 and 3.2 mgm., 1 and 2 hours respectively after tourniquet release.

DISCUSSION

A high unilateral tourniquet applied to the hind limb of a rat, maintained for about 5 hours and then released, leads to the production of shock which, without therapy, is almost constantly fatal. Of 75 such experimental rats, 73 died within 24 hours, a mortality of 92 per cent. The time of survival after release of tourniquet ranged from 2 to 23 hours, and averaged 7 hours. In 65 rats the amount of fluid lost into the injured extremity

at the time of death varied from 3.2 to 7 per cent, and the mean loss was 5 per cent.

Although blood pressures were not recorded in this study, the course of events prior to death was typical of shock. Within 1 or 2 hours after removal of tourniquet the animals usually began to show apathy, weakness and neuromuscular depression. These were progressive, and were followed by pallor and lividity of paws, prostration and respiratory distress, which became marked shortly before death.

Edema. The quantity of edema fluid in the injured extremity was determined by bisection of the animal, utilizing the technique of Blalock (1) modified by Hechter, Krohn and Harris (2). Gain in weight of injured over normal leg gave the amount of extravasated fluid. The method was used originally by Cannon and Bayliss (6) who severed injured and normal extremities across the upper thigh. Parsons and Phemister (7) compared the weights of the lower extremities, after symmetrical amputation along the lines of attachment to the innominate bones. Blalock's modification was designed to include groin, pelvis and flank on each side, since edema occurs in these regions as well as in leg proper. This undoubtedly gave a more accurate estimate of the quantity of extravasated fluid.

Bisection can be performed with a fairly high degree of accuracy. Cullen and Freeman (8) separated the extremities of 15 normal dogs and obtained an average difference in weight amounting to 0.32 per cent of body weight. In 12 normal rats we obtained an average error of only 0.18 per cent of body weight.

Moon's objection (9) to the use of bisection is based on the fact that as fluid escapes into the affected side, a simultaneous absorption occurs from the tissues of the normal side, thereby decreasing its weight. Since the difference in weight of the extremities includes twice the volume of the fluid shifted, *i.e.*, volume gained in one leg and lost from the other, he claims that the error of the method is doubled. Although this criticism is valid, the estimated error is too large. The extravasated fluid comes from the rest of the body as well as from the normal extremity, the latter probably contributing an amount roughly proportional to its weight. On this basis, the figures

for fluid loss obtained by bisection are about 10 per cent higher than the actual values.

In our study, rats with unilateral tourniquet shock showed a mean fluid loss of 5 per cent of body weight at the time of death, which averaged 7 hours after removal of tourniquet. Similar figures have been obtained for dogs in both tourniquet and traumatic shock by other investigators who also used the method of bisection. In Blalock's animals, fluid loss at death ranged from 4.1 to 5.1 per cent of body weight (1). Parsons and Phemister's values were approximately in the same range (7), while Holt and MacDonald (10) reported fluid loss slightly in excess of 4 per cent of body weight. In 13 dogs dead of tourniquet shock, Wilson and Roome (11) stated that the average increase in weight of the injured leg was 3.54 per cent of body weight. Ashworth, Jester and Guy (12) reported a value of about 4 per cent in shock produced by a combination of tourniquet and trauma. Perlow and co-workers (13) found a fluid loss of 4 to 6.1 per cent of body weight in dogs following occlusion of the veins to one hind extremity. Using a similar technique, Schlesser and Asher (14) obtained figures ranging from 3 to 8 per cent of body weight.

Fluid loss in shock has also been determined by the method of immersion (15 to 17). Swelling of the limb is obtained from the volume of fluid displaced before and after injury, and at death. This procedure fails to include edema fluid which collects in soft tissues, especially pelvis and flank, without causing expansion and hence gives values lower than those obtained with bisection. A comparison of the 2 methods in dogs by Green and associates (16) showed that the gain in volume measured by immersion ranged from 63 to 88 per cent of the difference in weight of the 2 extremities.

Nickerson (17) inserted dogs into a specially constructed tank and measured gain in volume of the traumatized extremity by the amount of water drained from the tank. He reported that in fatal cases the average swelling just after trauma was 4.1 per cent, and at death 4.8 per cent, of body weight.

The rats in this study showed reactive hyperemia of the constricted leg almost immediately after renewal of blood flow. Edema also appeared

promptly, and was often detected grossly in the paw within a few minutes. The entire leg then underwent marked swelling, and progressive increase in size was visible for about 1 hour following restoration of the circulation. It was plainly evident that a large quantity of fluid was lost rapidly into the tissues of the limb.

At first, the edema fluid collected principally in the leg below the site of constriction, and when further expansion here was prevented by tissue tension, there was overflow into the lower abdominal wall and flank. The fluid gravitated into the fascial plane between subcutaneous tissue and oblique abdominal muscles. As shown by the curve in Figure 1, fluid loss was very rapid during the first ½ hour after tourniquet removal, diminished during the next ½ hour, and then leveled off distinctly thereafter. The mean loss amounted to 2.8, 3.4, 4.2 and 4.8 per cent of total body weight, 30 minutes, 1 hour, 2 hours and 3 hours respectively after the tourniquet was released. Haist and Hamilton (18) obtained a similar curve in rats based on increase in volume of the hind limbs following release of constricting clamps. The progressive reduction in fluid loss is what might be expected in tourniquet shock, because of the limiting factors of falling blood pressure, increasing tissue tension, and high osmotic pressure of the extravasated fluid.

The extravasation of fluid is due to capillary injury resulting from the long period of total ischemia. Complete and prolonged anoxemia causes marked increase in capillary permeability (19, 20). Presumably this is brought about by the action of chemical substances liberated locally by tissue breakdown. Holt and MacDonald (10) state that there is no evidence that such substances enter the systemic circulation.

The edema fluid was usually pale pink, slightly viscid, and resembled plasma. Hemorrhage was minimal or negligible, as shown by gross and microscopic study. In this respect, tourniquet shock differs from traumatic shock in which there is usually considerable loss of blood in addition to plasma.

Total protein, determined by the falling drop method, ranged from 5 to 7 grams, which was approximately 68 to 80 per cent of the corresponding levels in blood serum. The high protein content of the fluid probably explains the lack of signifi-

cant change in serum protein despite marked hemoconcentration. In dogs with tourniquet shock, protein levels of the extravasated fluids have been reported at slightly less (12) than or approximately the same as in blood plasma (21). Similar results are given for the edema fluid in mild trauma (22), traumatic shock (23), burns (24), and in experimental freezing shock (25, 26).

Protein fractionation was not done in this study. However, Ricca and co-workers (23) stated that the albumin to globulin ratios of edema fluid from dogs dead of traumatic shock were higher than the corresponding ratios in serum. This was due to increase in albumin or decrease in globulin, or both. The authors calculated that much more protein, especially albumin, was present than could be accounted for by the loss from blood stream, and suggested that the extra amount originates as a result of local liberation of cell proteins from crushed muscle fibers. On the other hand, Ashworth, Jester and Guy (12) working with dogs in shock due to tourniquet and trauma, reported that the amount of protein lost locally was only slightly in excess of the decrease in plasma protein, *i.e.*, loss in the leg amounted to 34.2 per cent of the original circulating protein, while the loss from plasma was 32 per cent of the control value.

Hemoconcentration was a uniform observation in our experiments. Although poor correlation has been reported (27, 16) we obtained fairly constant changes in relation to magnitude of edema. Hematocrit levels rose sharply during the first 15 minutes following release of tourniquet, corresponding to the period of greatest fluid loss, less rapidly during the next 15 minutes, and leveled off somewhat abruptly thereafter. In the dog, aside from fluid loss, the initial rapid rise may be due partly to contraction of the spleen (21, 16) but whether this holds for the rat, whose splenic framework lacks a significant amount of smooth muscle, is uncertain. Cutaneous vasoconstriction is probably a contributing factor in the rat. In 65 rats the average hematocrit at death was 70, an increase of 25 points over the mean normal level. Terminally, the blood was dark, viscid, and only a very small amount, *i.e.*, about 1 ml. or less, could be obtained by decapitation.

In addition to capillary injury, interruption of

blood flow to an extremity for 5 hours leads to marked tissue breakdown, especially of muscle. Chemical decomposition, for example, is shown by rise in serum creatine on resumption of circulation. Bollman and Flock (28) found that total anoxemia of muscle for more than 3 hours resulted in almost complete and irreversible hydrolysis of phosphocreatine. When circulation was restored, resynthesis of organic compounds did not occur, and both creatine and inorganic phosphates were rapidly washed out by the blood. In our study, serum creatine levels 30 minutes after tourniquet release were increased approximately 100 per cent over the normal values, and 5 to 6 hours after release the increase was about 400 per cent. In 8 dogs with traumatic shock Duncan and Blalock (29) found that the plasma creatine levels 4 to 6 hours after trauma were considerably elevated in 3 animals, and slightly elevated in 5 animals.

Progressive degeneration of muscle was apparent from microscopic sections made at intervals after renewal of blood flow. There were disruption and fragmentation of fibers, separation into longitudinal fibrils, loss of transverse striations, and areas of granular degeneration. Some fibers were deeply acidophilic or basophilic, while others showed pale staining foci. Nuclear alteration included pyknosis and lysis. Changes were sometimes evident as early as 15 minutes after circulation was restored, and were well developed 2 or 3 hours later. In rats dead from 12 to 20 hours after removal of tourniquet, some muscle fibers showed hyaline necrosis.

Autopsies on control rats which died in shock revealed marked hyperemia of the mesenteric veins. Most organs were dark red, hyperemic and not excessively moist. No free fluid was found in the serous cavities. Microscopically there was generalized hyperemia of viscera involving capillaries and venules. Capillary hemorrhage was rare and usually absent. Focal necrosis and significant cellular exudate were not observed. There was no evidence of widespread tissue edema and pulmonary edema was notably absent.

Parenchymal organs such as liver, adrenal and kidney (especially the first), showed alterations in morphology of the epithelial cells. These were usually reduced in size, irregular in shape, and sometimes appeared shrunken. Cytoplasm was condensed, more deeply acidophilic, homogeneous, and revealed loss of the normal foamy or granular appearance. Nuclei were often smaller than usual, and pyknotic. In the liver, such changes were commonly diffuse, although somewhat more distinct in the central zones of the lobules. While the significance of this lesion is uncertain, it may be related to the loss of intracellular fluid and other components in shock.

Autopsy studies of rats sacrificed at various time intervals after tourniquet removal indicated progressive development of the lesions observed in the control animals. As a result, it was generally possible to distinguish between rats sacrificed 15 or 30 minutes after release of constriction and those sacrificed at the end of 2 or 3 hours.

In these experiments, the magnitude and rapidity of local fluid loss are sufficient *per se* to explain the development of shock. Experimentally, comparable loss of blood (30 to 32) or plasma (33, 34) to the exterior results in shock. In the rat, shock is produced by withdrawal of whole blood corresponding in amount and rate to fluid loss by tourniquet (35). From the standpoint of circulatory dynamics, the large extravasation of fluid from the blood stream into the tissues of the hind limb leads to decrease in circulating blood volume, followed by diminished venous return to the heart, reduction in cardiac output and fall in blood pressure (36 to 39). Actual determinations have shown that the reduction in blood volume which accompanies both tourniquet and traumatic shock can be accounted for entirely by the quantity of fluid lost in the injured area (40, 17, 12). The bulk of evidence now favors the view, that, in these forms of shock, increased capillary permeability and loss of fluid are local rather than widespread (41, 40, 34).

Although there is convincing evidence that shock is initiated by local fluid loss, secondary or sustaining factors undoubtedly play a rôle in its maintenance. These apparently arise in connection with the metabolic disorders resulting from prolonged tissue anoxia. Recent studies (42, 43) indicate that a humoral vasodepressor principle originating in liver and skeletal muscle occurs in the blood in the later stage of shock. Such depressor material tends to eliminate compensatory peripheral vascular mechanisms in

shock, and hence may be a significant factor in the development of irreversibility.

CONCLUSIONS

1. Fatal tourniquet shock in the rat is associated with marked extravasation of fluid into the injured hind limb.

2. The fluid loss amounts to 2.1 per cent of body weight 15 minutes after tourniquet release, and to 2.8, 3.4 and 4.2 per cent of body weight 30 minutes, 1 hour and 2 hours respectively after release.

3. The magnitude and rapidity of the fluid loss adequately explain the origin of shock. Withdrawal of whole blood in comparable amount and at the same rate results in shock.

4. The development of shock is accompanied by marked hemoconcentration and rise in serum creatine. There is no significant change in total serum protein.

5. Morphologic study of rats dead of tourniquet shock reveals generalized hyperemia of viscera involving capillaries and venules. Capillary hemorrhages are rare and widespread tissue edema does not occur.

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STUDIES ON *LEPTOSPIRA ICTEROHAEMORRHAGIAE*. II. A CRITICAL STUDY OF THE EFFECT OF PENICILLIN ON *LEPTOSPIRA ICTEROHAEMORRHAGIAE IN VITRO* AND IN LEPTOSPIROSIS IN GUINEA PIGS

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Observations on the therapeutic effect of penicillin in both experimental and clinical leptospirosis have not been in full agreement. Heilman and Herrel (1) reported that of 32 guinea pigs which, 17 to 24 hours after the inoculation with *L. icterohaemorrhagiae*, received a total of 800 oxford units (hereafter abbreviated as o.u.) of penicillin daily for 7 days, 23 showed a complete suppression of leptospirosis, while 9 gave evidence of relapse 3 to 7 days after treatment. These 9 animals became normal after an additional 3 to 4 days of penicillin treatment.

On the other hand, Augustine, Weinman, and MacAllister (2) of this Department found that a course of penicillin, begun after the appearance of jaundice and amounting to 24,000 o.u. daily for about 3 days, failed to alter the fatal course of the infection in 5 guinea pigs. In another group of 4 guinea pigs in which the treatment, consisting of 30,000 o.u. daily for 2 days, was begun 38 hours after inoculation, none was patently ill, although 3 died between the 7th and 11th days after inoculation. At autopsy these 3 animals showed no severe lesions, but leptospirae were found in the liver and kidneys. These authors (Augustine *et al.*) thought that the death of the 5 animals in the first group might have been due to penicillin toxicity, and that penicillin might have some suppressive, but not curative, effect in leptospirosis.

Working with *L. icterohaemorrhagiae* in mice and *L. canicola* in hamsters, Larson and Griffiths (3) reported that the administration of penicillin to a total of 400 o.u. per mouse, and 1600 o.u. per hamster, produced a marked beneficial effect when the treatment was begun 48 to 52 hours after inoculation with leptospirae, less favorable results after 66 to 78 hours, and no apparent effect after 88 hours. These results suggest that the success of penicillin therapy in leptospirosis depends on its early administration.

Alston and Broom (4) found that the presence of 0.4 to 50 o.u. of penicillin in 3.5 ml. of culture medium stopped the multiplication of both *L. icterohaemorrhagiae* and *L. canicola* introduced in a rich inoculum. The addition of as much as 240 o.u. of penicillin per 10 ml. of a well grown culture of *L. icterohaemorrhagiae* greatly reduced the number of leptospirae after 12 days of incubation as compared with a control culture, although motile leptospirae were present even after 3 weeks of incubation. On this observation, they based their belief that penicillin has some lethal effect on leptospirae. Alston and Broom (4) also confirmed the results of Heilman and Herrel (1) that penicillin treatment, if started 18 hours after inoculation with leptospirae, exerted a favorable therapeutic action in guinea pigs. It is worth mentioning that these authors (4) observed no suppressive effect of penicillin on the development of serum antibodies in the treated animals.

Cross (5) infected 2 guinea pigs with *L. icterohaemorrhagiae*, and treated 1 animal 6 days after inoculation with 3000 o.u. of penicillin daily for 4 days. The treated animal showed no illness, whereas the untreated one died of leptospirosis 10 days after inoculation.

Information on the therapeutic effect of penicillin in human leptospirosis has been very scanty, and very few cases have been studied. Hart (6) reported a case of leptospirosis which recovered spontaneously, although the patient discharged leptospirae in the urine. Upon administration of 90,000 o.u. of penicillin daily for 3 days, the number of leptospirae in the urine diminished on the 2nd, and the organisms disappeared on the 3rd day of treatment. Cross (5) noted the recovery of a patient with leptospirosis after a combined treatment of 20 ml. of antiserum on the 9th, 10th, and 11th days of the illness, and 120,000 o.u. of penicillin daily beginning on the 14th day, and

continued until a total of 800,000 o.u. were given. Carragher (7) reported favorable response of another case of leptospirosis to penicillin therapy which was started on the 7th day of the illness, and consisted of 160,000 o.u. daily for 4 days. Bulmer (8) summarized the results of 39 cases of leptospirosis among British troops in Normandy, of which 16 were treated with penicillin at 240,000 o.u. daily to a total of about 1 million o.u. One among the treated cases died of uremia, as against 2 among the 23 untreated cases. The author (8) noted that the penicillin treatment produced marked symptomatic improvement in most of the cases that recovered, but its value in reducing the mortality rate is doubtful because of the fact that the treatment has generally been begun too late in the course of the disease to change the prognosis of fatal cases. Hutchison *et al.* (9) reported another group of 17 cases of leptospirosis among British troops in Italy, in which 6 received, from the 6th to the 10th day of illness, 120,000 o.u. of penicillin daily to a total of about 600,000 o.u., 3 received antiserum, and 8 remained untreated. One among the penicillin-treated, 1 among the serum-treated, and 2 among the untreated patients died. None of the other 5 who recovered in the penicillin-treated group showed evidence of benefiting from the penicillin.

In view of the present confused state of knowledge about the therapeutic value of penicillin in leptospirosis, the author attempted to study the nature of the activity of penicillin on *L. icterohaemorrhagiae* both *in vitro* and in guinea pigs, to find the minimum effective dose of penicillin in suppressing the disease process in guinea pigs, and to determine how far the disease may proceed before it is too late for the penicillin to modify its course.

MATERIALS

The strain of *L. icterohaemorrhagiae* used in this study was secured from the National Institute of Health, Bethesda, Maryland, through the Health Department of Boston, Massachusetts. It was maintained in culture in a fluid leptospira medium described previously (10). This strain, after several transfers, was of low virulence to experimental animals, and was used only in *in vitro* tests.

A virulent substrain of *L. icterohaemorrhagiae* was isolated in culture from a diseased guinea pig inoculated with the above mentioned strain of leptospira in a semisolid leptospira medium also described in the previous

paper (10). The virulence of this substrain was maintained in culture by a method described in the same paper.

As described previously (10), *L. icterohaemorrhagiae* grows prosperously in both media, and reaches the peak in about 2 weeks of incubation at 23° to 26° C., at which the leptospira density may be as high as 60 million per ml. of culture. The virulent substrain, when inoculated in 0.5-ml. amounts intraperitoneally, in young guinea pigs (weighing 250 to 300 grams), almost always produced a fatal leptospirosis. Jaundice usually appeared on the 3rd or 4th day after inoculation.

The sodium salt of penicillin was used in this study, and was obtained commercially. A solution containing 5000 to 10,000 o.u. per ml. of solution was prepared in sterile distilled water shortly before use. The potency of each solution was tested against a culture of *Staphylococcus aureus*. The solution was kept at 2° C. when not in use, and was discarded after 48 hours' storage.

The procedures used in the different tests are described, and the results observed are presented and discussed, under the following separate headings.

Activity of penicillin on L. icterohaemorrhagiae in water

In order to understand better the effect of penicillin in leptospirosis, it is desirable to know precisely how penicillin acts on the leptospirae. Tests were, therefore, made first to determine the survival of leptospirae in water containing varying amounts of penicillin at various temperatures and lengths of storage.

Suspensions of leptospirae in water were prepared by centrifuging a 2- to 3-weeks-old culture of *L. icterohaemorrhagiae* in the fluid medium at 4000 r.p.m. for 30 minutes, washing once, and resuspending the sediment in sterile tap water. The suspension thus prepared usually had about 20 million organisms per ml. of water, about 90 per cent of which were motile. For the method of leptospira count, see the previous paper (10). Two ml. amounts of suspension were dispensed in a series of sterile small tubes, 10 mm. in diameter. Dilutions containing 10, 100, 1000, and 10,000 o.u. of penicillin in sterile distilled water were prepared from the stock solution. Two ml. of each of these 4 dilutions were added to the tubes of leptospira suspension, and mixed. One tube of leptospira suspension without penicillin served as control. Of 3 sets of tubes thus prepared, 1 set was placed in a cold room at 10° C, 1 set at room temperature (23° C. ± 1), and 1 set in an incubator at 37° C.

These tubes were examined by darkfield illumination for motile leptospirae every other day for a period of 2 weeks. In each examination, a

total of about 100 organisms were counted, and the number of motile ones was expressed as percent of the total. The results thus obtained are presented in Table I.

Before discussing the data in Table I, it must be noted that in a separate study made on the survival of L. *icterohaemorrhagiae* in tap and Charles River waters, and in tap water containing 10 per cent sewage (11), it was found that although most of the leptospirae survived only the first few days of storage, a small number of them was capable of multiplying at very slow rate for a few weeks or more, depending on the temperature and the amount of nutritive substance in the water. While much of the nutrients of the culture was removed during the preparation of the suspension, a small remaining amount seemed to furnish sufficient food for the multiplication of a few leptospirae. Temperature seemed to affect the survival of leptospirae in several ways. In the presence of bacterial contamination, the higher temperatures favor the growth of bacteria which are detrimental to the survival of leptospirae. In the absence of bacterial contamination, the higher temperatures favor the rate of multiplication of leptospirae, but shorten the survival time of the individual leptospira which is no longer multiplying.

In the present study, the same phenomenon was noticed. As shown by Table I, in the controls a large per cent of the leptospirae became nonmotile in the first few days of storage. The decrease in the number of motile organisms is shown to be lowest at 10° C., higher at 23° C., and highest at 37° C. However, the most important phenomenon brought out by the data in this table is that, with the presence of penicillin in amounts of from 5 to 5000 o.u., the percentages of motile leptospirae were not significantly different from that in the control up to the 12th day of storage at 10° C., the 8th day at 23° C., and the 4th day at 37° C. After these periods of storage, the controls showed slightly, but consistently, higher percentages of motile organisms than the penicillin-treated. It must also be noted that while a few of the motile leptospirae in the controls showed evidence of multiplication (fission of long leptospirae was observed during microscopic examination) none of the penicillin-treated tubes showed such dividing forms. These results suggest that although the penicillin has no leptospiricidal effect even at high concentrations, it seems to prevent the reproduction of the leptospirae.

Activity of penicillin on L. icterohaemorrhagiae in culture

While the results of the above experiments clearly demonstrate that, *in vitro*, penicillin is not appreciably leptospiricidal, it is indicated that the drug may have an inhibitory effect on the reproduction of leptospirae. The following experiments were performed to gain information on this very question.

Each of a series of flasks of the fluid leptospira medium in 25 ml. amounts was seeded with 1 ml. of a well mixed, 2-weeks old culture of L. *icterohaemorrhagiae*. The initial leptospira density of the seeded medium in each flask was ascertained by the same counting method as described in the previous paper (10).

The seeded flasks were grouped into 3 sets, with 7 flasks in each set. Into each 6 flasks was added separately a 1-ml. amount of the penicillin solutions, containing 5, 10, 20, 50, 250, and 1250 o.u. The final penicillin concentra-

TABLE I

Survival of L. icterohaemorrhagiae in water containing varying amounts of penicillin

No. of days stored	O.U. of penicillin	At 10°C					At 23°C					At 37°C				
		0	5	50	500	5000	0	5	50	500	5000	0	5	50	500	5000
						percentage of motile leptospira										
At start		96	97	92	96	92	94	90	97	91	95	98	93	91	95	95
2		64	72	60	65	61	46	43	36	41	39	20	24	19	22	21
4		55	62	54	75	50	37	33	28	35	30	15	11	13	15	10
6		43	40	35	36	39	28	25	22	26	25	5	2	1	2	1
8		28	26	29	31	25	15	11	14	9	10	3	0	0	0	0
10		21	19	22	19	17	11	5	5	8	6	1	0	0	0	0
12		17	14	16	13	16	10	3	4	3	5	1	0	0	0	0
14		13	6	4	5	5	8	2	2	1	2	0	0	0	0	0

TABLE II

Leptospirostatic effect of penicillin in culture

No. of days stored	Penicillin o.u. per ml.	0	0.2	0.4	0.8	2.0	10.0	50.0
		number of leptospira per ml. of culture						
At 10°C								
Initial		8.1×10^5	7.8×10^5	8.5×10^5	7.3×10^5	7.5×10^5	8.0×10^5	8.3×10^5
7		9.2×10^5	8.6×10^5	5.9×10^5	5.4×10^5	5.7×10^5	6.1×10^5	5.8×10^5
14		1.3×10^6	1.1×10^6	1.8×10^5	1.9×10^5	1.1×10^5	1.5×10^5	1.1×10^5
21		2.4×10^6	1.8×10^6	3.8×10^4	3.7×10^4	3.2×10^4	3.5×10^4	2.8×10^4
28		2.9×10^6	2.2×10^5	1.2×10^4	1.4×10^4	1.8×10^4	1.8×10^4	1.1×10^4
35		3.1×10^6	2.5×10^5	1.0×10^4	1.0×10^4	1.0×10^4	1.0×10^4	1.0×10^4
At 23°C								
Initial		7.6×10^5	8.0×10^5	7.2×10^5	7.9×10^5	8.2×10^5	7.5×10^5	7.8×10^5
4		2.0×10^6	1.7×10^5	4.6×10^5	4.0×10^5	4.4×10^5	3.8×10^5	4.3×10^5
8		5.0×10^6	4.5×10^6	1.8×10^5	1.5×10^5	1.4×10^5	2.0×10^5	2.1×10^5
12		1.5×10^7	1.1×10^7	5.3×10^4	5.1×10^4	4.5×10^4	3.9×10^4	4.1×10^4
16		3.2×10^7	2.4×10^7	3.4×10^4	2.5×10^4	1.9×10^4	2.1×10^4	1.7×10^4
18		3.4×10^7	2.8×10^7	4.3×10^4	1.0×10^4	1.0×10^4	1.0×10^4	1.0×10^4
At 37°C								
Initial		8.6×10^5	8.2×10^5	7.6×10^5	8.0×10^5	7.9×10^5	7.5×10^5	7.8×10^5
2		2.7×10^6	2.2×10^6	2.5×10^5	2.8×10^5	2.1×10^5	2.2×10^5	2.6×10^5
4		5.6×10^6	5.1×10^6	8.7×10^4	9.2×10^4	8.5×10^4	9.0×10^4	8.4×10^4
6		1.1×10^7	8.8×10^6	4.5×10^4	2.3×10^4	1.8×10^4	1.5×10^4	1.4×10^4
8		7.8×10^6	6.8×10^6	4.7×10^4	1.0×10^4	1.0×10^4	1.0×10^4	1.0×10^4
10		4.8×10^6	5.6×10^6	5.0×10^4	1.0×10^4	±	±	±
12		3.5×10^6	3.2×10^6	4.8×10^4	±	−	−	−

tions were therefore 0.2, 0.4, 0.8, 2.0, 10.0, and 50.0 o.u. per ml. of the medium. The untreated flask served as a control. One set of flasks was held at 10° C., 1 set at room temperature (23° C. ± 1), and 1 set at 37° C. The leptospira density in each flask in each set was determined at scheduled time intervals by the same procedure previously described (10). The results thus obtained are summarized in Table II. Since many leptospirae were found dead in later periods of storage in the penicillin-treated cultures, the figures presented in the table include only the motile organisms.

Before analyzing the data in Table II, it should be noted that *L. icterohaemorrhagiae* differed from ordinary vegetative bacteria in that it multiplied at such a slow rate in artificial media that the increase in number of organisms was measureable only at intervals of days (11). The higher incubation temperature increased the growth rate, but at 37° C. the increase was observed only in the first few days, after which the growth dropped steadily. At 10° C. the growth increase was barely measureable at weekly intervals. This slow growth rate made the study of the leptospirostatic effect of penicillin difficult, since significant differences in the number of leptospirae between the treated and control flasks was not noticeable until after several days of incubation at room or body tem-

perature, and after several weeks at low temperatures. Deterioration of penicillin would have taken place during the period of observation.

In spite of the difficulty, the results indicate that penicillin has a definite leptospirostatic effect on *L. icterohaemorrhagiae*. Since penicillin is relatively stable at 10° C. or below, the results obtained at 10° C. may be interpreted without seriously considering the matter of deterioration of the penicillin. As shown in the first part of Table II, multiplication of leptospirae was stopped in the culture containing 0.4 o.u. of penicillin per ml., and the culture containing 0.1 o.u. showed an increase in the number of leptospirae not significantly different from that of the control. It is of importance to note again that further increase in the dosage of penicillin, even to as much as 50 o.u. per ml., produced no significant change in leptospirostatic effect. The data also indicate that at 10° C. the majority of leptospirae survived less than 2 weeks, some survived less than 3 weeks, and a small number survived more than 4 weeks in the medium where penicillin exerted a leptospirastatic effect.

The results at 23° and 37° C. followed, in general, the same pattern as those of 10° C., except

that the differences in the number of leptospirae were much greater between cultures with and without the leptospirostatic effect of penicillin. These accentuated differences at higher temperatures are attributed to the fact that the leptospirae multipled at greater speeds, and that, when they failed to multiply as under leptospirostatic effect, they survived a shorter time at these temperatures. It is also noteworthy that the culture containing 0.4 o.u. of penicillin per ml. showed a slight increase in the number of leptospirae after a steady decrease in 12 days of incubation at 23° C. A similar result was observed in the culture containing the same amount of penicillin after 4 days of incubation at 37° C. This phenomenon is explained on the basis that the amount of penicillin present in these cultures might have deteriorated to a level at which it no longer inhibited reproduction.

A determination of the residual penicillin by the method described by Fleming (12) was made

TABLE III

Deterioration of Penicillin in Cultures of L. icterohaemorrhagiae

Incubation temp.	Days of incubation	Amount of penicillin in o.u. per ml. of Culture					
		Initial	Residual	Initial	Residual	Initial	Residual
23°C	16	0.4	0.1	0.8	0.4	2.0	0.5
37°C	6	0.4	<0.1	0.8	0.2	2.0	<0.5

on the 16th day of incubation at 23° C., and on the 6th day at 37° C. on the cultures containing initial amounts of 0.4, 0.8, and 2.0 o.u. of penicillin per ml. The results, as presented in Table III, showed that roughly 50 to 75 per cent of the penicillin had deteriorated.

Summing up these results, one may conclude that penicillin is definitely leptospirostatic in culture, and that in order to obtain a leptospirostatic effect, a minimum dosage of 0.4 o.u. of penicillin per ml. of medium is necessary. No advantage is gained by increasing the amount of penicillin, pro-

TABLE IV

Effect of penicillin on L. icterohaemorrhagiae in guinea pigs

Group no. of guinea pig	Penicillin	Cultural results and serum penicillin levels at stated days after inoculation										Remarks*
		1		3		5		7		9		
		NPC[1]	SPL[2]	NPC	SPL	NPC	SPL	NPC	SPL	NPC	SPL	
I	o.u. per day 0	6/6	0	6/6	0	4/4	—	2/2	—	—	—	1 died on the 5th, 1 on the 7th, and 1 on the 8th day
II	100	6/6	<0.1	6/6	<0.1	6/6	<0.1	4/4	—	2/2	—	1 died on the 7th, 1 on the 8th, and 1 on the 9th day
III	200	6/6	0.1	6/6	>0.1	6/6	0.1	5/6	—	3/4	—	1 died on the 9th, and 2 died on the 10th day
IV	400	6/6	>0.1	6/6	>0.1	5/6	<0.2	5/6	—	3/4	—	1 died on the 9th, 1 died on the 11th day, 1 sick on the 12th day and recovered
V	600	6/6	>0.2	4/6	>0.2	2/6	>0.2	0/6	—	1/6	—	1 sick on the 10th day and died, 1 sick on the 13th day and recovered, 1 normal
VI	800	6/6	0.4	2/6	>0.4	0/6	0.4	0/6	—	0/6	—	1 sick on 13th day and recovered, 2 normal
VII	1,000	6/6	>0.4	3/6	>0.4	0/6	>0.4	0/6	—	0/6	—	1 sick on 14th day and recovered, 2 normal
VIII	5,000[4]	6/6	0.6	2/6	0.2	0/6	<0.1	1/6	—	3/6	—	1 sick on 10th day, 2 sick on 11th day, 2 died, 1 recovered

[1] NPC = Number of cultures positive for leptospira.
[2] SPL = Serum penicillin level in o.u. per ml. of serum.
[3] All animals that died showed jaundice before death, and typical haemorrhages and leptospirae in liver at autopsy.
[4] Single dose given together with the infective inoculum.

vided the 0.4 o.u. per ml. level is maintained during the surviving period.

Effect of Penicillin on L. icterohaemorrhagiae in Guinea Pigs

Having observed the leptospirostatic effect of penicillin in culture, a study was then made of the effect of penicillin on L. *icterohaemorrhagiae* in guinea pigs.

Tests were made in 24 animals weighing 250 to 300 grams. Since it is essential in these tests to have a sufficient number of leptospirae in the blood of inoculated animals to give positive cultures even with a small inoculum, each animal received intraperitoneally 5 ml. of a 2-weeks old culture of the virulent substrain of L. *icterohaemorrhagiae*, containing about 40 million organisms per ml. These animals were divided into 8 groups of 3 individuals. Each animal of 6 groups received, 12 hours after inoculation, penicillin at dosages of 50, 100, 200, 300, 400, and 500 o.u. twice daily for a period of 7 days respectively. One group of animals received a single dose of 5000 o.u. of penicillin per animal shortly after the inoculation. The last group received no penicillin, serving as control.

Blood samples of 0.2 ml. amounts were taken by cardiac puncture from all animals 1, 3, 5, 7, and 9 days after inoculation. One of the 3 samples from each group was used for determining the serum level of penicillin, and the other 2 were inoculated in 0.1 ml. amounts into the 6 flasks of semisolid leptospira medium, and incubated at room temperature. The dilution of the blood in the medium was so high (0.1:25) that the amount of penicillin carried into the medium was far below the level necessary to exert leptospirostatic effect. The results thus obtained are summarized in Table IV.

As shown in Table IV, with a daily dosage of 600 to 800 o.u. of penicillin per animal, no leptospirae were recovered from the 0.3 ml. of blood taken 5 days after inoculation, when the serum penicillin level was between over 0.2 to 0.4 o.u. per ml. The results obtained with a daily dose of 1000 o.u. of penicillin were essentially the same as those with 800 o.u., confirming the former observation on cultures that no advantage is gained by increasing the dosage of penicillin when a leptospirostatic level has been reached. However, the fact that 2 of the 3 animals in Group V showed relapse in 4 to 6 days, and that 1 individual in each of Groups VI and VII relapsed 6 to 7 days after the last dose of penicillin, suggests that some leptospirae may have penetrated into the liver parenchyma in the early days after inoculation, where the amount of penicillin might have failed to reach a leptospirostatic level. When the serum penicillin dropped to sub-leptospirostatic level after the treatment was stopped, multiplication of the leptospirae was resumed and disease produced. It is of interest to note that most of the animals suffering from relapse recovered spontaneously, suggesting that some immunity had already been acquired by these animals which was able to stem the natural course of the disease.

To confirm these assumptions, 6 guinea pigs (weighing 250 to 300 grams) were inoculated with the virulent substrain of L. *icterohaemorrhagiae* in a similar way as before. Four animals received, beginning 12 hours after inoculation, 400 o.u. of penicillin twice daily, and 2 re-

TABLE V

Effect of penicillin on L. icterohaemorrhagiae *in blood and liver of, and on antibody response in, guinea pigs*

Guinea pig no.	Peni-	No. of positive blood cultures					Serum penicillin level			Leptospira in liver at autopsy		Agglutination titer of sera			
		days after inoculation					*days after inoculations*			D.E.*	Culture	*days after inoculation*			
		1	3	5	7	9	3	5	7			7	11	15	19
	o.u. per day						o.u. per ml.								
1	800	2/2	1/2	0/2	0/2		0.2	0.4	0.4	—†	—				
2	800	2/2	1/2	1/2	0/2	0/2	0.4	0.2	0.4	—	—				
3	3,000	2/2	1/2	0/2	0/2		0.8	0.6	1.0	—	—				
4	3,000	2/2	1/2	0/2	0/2	0/2	0.6	0.6	0.8	—	—				
5	800	2/2	1/2	0/2	0/2	0/2						$\frac{1}{40}$	$\frac{1}{160}$	$\frac{1}{320}$	$\frac{1}{640}$
6	800	2/2	1/2	0/2	0/2	0/2						$\frac{1}{20}$	$\frac{1}{80}$	$\frac{1}{160}$	$\frac{1}{320}$

* D.E. = Direct examination in darkfield.
† Although the livers from the first 4 animals were positive for living leptospirae, the numbers found in direct examination were very small, much smaller than found in ordinary untreated infected animals.

ceived 1500 o.u. twice daily. Penicillin administration was continued for 7 days. Blood was taken and cultured for leptospira as before. The serum penicillin was again determined in 2 of the 4 animals that received 800 o.u. daily, and in the 2 that received 3000 o.u. on the 3rd, 5th, and 7th day after inoculation. One in each 2 of these animals was sacrificed on the 9th day, and the other 2 on the 11th day after inoculation. From each animal a piece of liver was removed and emulsified in sterile normal saline and examined for living leptospirae directly, by darkfield, and by cultures. From the other 2 animals that received 800 o.u. daily of penicillin, blood samples were taken on the 7th, 11th, 15th, and 19th day after inoculation. The sera were tested for agglutinins with a suspenion of formolized *L. icterohaemorrhagiae*. The results thus obtained are presented in Table V.

From Table V it is shown that, as noted before, leptospirae in the blood of guinea pigs were no longer recovered in culture after a period of about 5 days under the leptospirostatic effect of penicillin. However, even with a daily dose of 3000 o.u. of penicillin, resulting in a serum penicillin level of over 0.6 to 1.0 o.u. per ml., living leptospirae were still found in the liver 9 to 11 days after inoculation, or 6 to 8 days after the blood yielded negative cultures. It is too complicated to determine the tissue penicillin level in the liver; but it would seem either that penicillin failed to reach a leptospirostatic level in the liver in spite of the high serum penicillin level, or that the liver parenchyma has some penicillin-neutralizing power, thus offering a haven for small numbers of leptospirae. In this connection, it is interesting to note that Augustine *et al.* (2) found living leptospirae in the liver of the 3 guinea pigs that had received 30,000 o.u. of penicillin 38 hours after inoculation daily for 2 days, and had died of apparent penicillin toxicity on the 7th, 8th, and 11th days.

The findings on the agglutination titre of the sera from guinea pigs Nos. 5 and 6 are in agreement with that of Alston and Broom (4) that the penicillin adminstration did not prevent the production of agglutinating antibodies, although the titres were not as high as would be observed in ordinary infection. This moderate antibody production is thought to be a response to the large number of leptospirae which failed to multiply, and died in the first few days after inoculation, and is held responsible for the spontaneous recovery of the animals that underwent relapse.

Effect of penicillin in leptospirosis in guinea pigs

Having observed the leptospirostatic effect of penicillin on *L. icterohaemorrhagiae* in guinea pigs, a study was finally attempted of the therapeutic effect of penicillin in leptospirosis in guinea pigs, particularly of the effect at various stages of the disease process.

Twenty young guinea pigs (weighing 250 to 300 grams) were inoculated in the same way as in the 2 previous tests with the virulent culture of *L. icterohaemorrhagiae*. These animals were divided into 5 groups. Four groups received 500 o.u. of penicillin twice daily for a period of 7 days (unless death occurred during treatment) starting 3, 4, 5, and 6 days after the inoculation. The last group was not treated, and served as a control. The results recorded in this experiment are shown in Table VI.

The results presented in Table VI, together with those in Tables IV and V, show clearly that peni-

TABLE VI

Effect of penicillin treatment on leptospirosis in guinea pigs

Group no. of guinea pig	Penicillin	Time lapse between inoculation and treatment	Condition of animals at the time when treatment began	Results of penicillin treatment*
	o.u. per day	*days*		
1	1,000	3	All showed loss of appetite, 3 with roughness of fur, 2 with slightly yellow tinged ears	The 2 showing slight jaundice died on the 6th and 7th days. The other 2 recovered without showing apparent jaundice
2	1,000	4	All were sick with moderate jaundice	All died, 1 on the 6th, 3 on the 7th day after inoculation
3	1,000	5	All very sick, 3 with intense jaundice	All died, 2 on the 6th, 2 on the 7th day after inoculation
4	1,000	6	One died, 2 very sick as in Group 3	All 3 died on the 7th day after inoculation

* All dead animals were autopsied and showed typical haemorrhages and large numbers of living leptospirae in their livers.
All 4 animals in control group died of the disease 4 to 5 days after inoculation.

cillin, through its leptospirostatic effect, suppresses the development of leptospirosis, when it is administered during the incubation period and in amount adequate to maintain a serum level of about 0.4 o.u. per ml. for a period of 7 days. The therapeutic effect may still be observed if penicillin is given before jaundice occurs. After the appearance of jaundice, the effect of penicillin is insignificant. This observation is, in general, in agreement with that of Larson and Griffitts (3), and confirms the statement made by Augustine *et al.* (2) that penicillin may have suppressive effect in leptospirosis if it is given before symptoms occur.

It must be noted that the 2 animals in Group 1 that recovered when penicillin was given shortly after the animals became sick, showed definite improvement on the 2nd day of treatment, and returned to normal on the 3rd day. Those animals that died in spite of the treatment, showed no apparent improvement in symptoms. This observation seems to support the statement made by Bulmer (8) that penicillin treatment in human leptospirosis resulted in symptomatic improvement in cases that were to recover, but was generally introduced too late to change the course of fatal cases. It would seem that in infections in which the liver is involved, death is a result of the liver damage which may not be checked or improved, even when the causative agent has been brought under control.

SUMMARY AND CONCLUSIONS

A study has been made of the effect of penicillin on *L. icterohaemorrhagiae* in water, in culture, and in guinea pigs, and also on the therapeutic effect in leptospirosis in guinea pigs. In this study, the following points have been observed.

1. Penicillin in an amount of about 0.4 o.u. per ml. or more, *in vitro*, exerted a leptospirostatic effect, but showed no leptospirocidal effect even at concentrations as high as 5000 o.u. per ml. Being unable to multiply, the leptospirae survived a limited number of days only, more days at low temperatures, and fewer days at higher temperatures.

2. The leptospirostatic effect was also observed in guinea pigs as evidenced by the disappearance of leptospirae from the blood 3 to 5 days after inoculation, when a daily dosage of about 800 o.u. and a serum level of over 0.2 o.u. per ml. were

maintained. This, however, did not clear the liver of leptospirae. No advantage was obtained by increasing the dose to 3000 o.u. daily.

3. Some guinea pigs in which the development of leptospirosis was suppressed by early administration of penicillin relapsed several days after the last dose of penicillin, but recovered spontaneously in most cases. The recovery was attributed to the partial immunity developed in response to the large number of organisms inoculated. Agglutination tests made showed that fair amounts of antibodies were produced, in spite of the fact that penicillin suppressed the development of leptospirosis.

4. Penicillin seemed to have some therapeutic effect in infected guinea pigs if the treatment was introduced before the appearance of jaundice. After the appearance of jaundice, none of the animals tested appeared to benefit by the treatment.

From these observations, it is concluded that (1) penicillin has no leptospiricidal effect, but (2) has a leptospirostatic effect both *in vitro* and *in vivo* when a concentration of about 0.4 o.u. of penicillin per ml. is maintained, and (3) that the therapeutic effect of penicillin in leptospirosis in guinea pigs depends on its administration before the appearance of jaundice or, in other words, before the liver is seriously damaged. Judging from the data on the dosage and serum level of penicillin necessary to produce a leptospirostatic effect in guinea pigs, it is estimated that a daily dose of 250,000 to 300,000 o.u. should be administered to human cases to show a leptospirostatic effect. In view of the fact that leptospirosis in young guinea pigs is a much more serious disease than that in human beings, these results should *not* discourage the use of penicillin in human cases, even though the treatment is usually started after symptoms have developed.

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QUANTITATION AND REGIONAL DISTRIBUTION OF SWEAT GLANDS IN MAN [1]

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The distribution of sweating in normal and pathological conditions has been the subject of many investigations, and many methods for qualitative and quantitative measurement of the amount of sensible and insensible perspiration have been described. A survey of available literature has revealed fewer methods for quantitation of the number of functionally active sweat glands, and none which combine the technical simplicity and accuracy of the method herein described.

Of the so-called colorimetric methods, Minor's classical starch-iodine test (1) is the most commonly used. This method is not useful in quantitative studies however, and is subject to clinical objection, because of the necessity of application of a starch paste with a resultant black stain in a positive test. Roth (2) reported a clinical test for sweating in which active secretion is marked by a color change in cobalt chloride on the skin, and Silverman and Powell (3) report a colorimetric test involving a reaction between ferric chloride and tannic acid in the presence of active sweating. Gurney and Bunnell (4) described a method in which paper is floated over silver nitrate solution and exposed to ultraviolet light; sweating is indicated by small brown spots of reduced silver nitrate. Direct microscopic examination of active sweat pores is reported by Kuno (5), and by Lobitz and Osterberg (6), and Buley (10).

METHOD

The method described below involves the reaction of starch and iodine, but eliminates the necessity of applying a coating of starch powder over the skin. A dilute solution of iodine (2 to 3 per cent in 95 per cent alcohol) is painted over the area and allowed to dry. (For quantitative purposes we use a blank rubber stamp, 1 sq. cm. in area, with which the iodine can be stamped on the area to be studied, thus providing a convenient and constant area in which to count the active glands.) A blank piece of ordinary bond paper is then pressed lightly over the

area for 20 seconds. Although any starch-containing paper may be suitable, we have found that the smooth hard finish of number 13 Voucher Bond contains sufficient starch, yet prevents excessive diffusion, and produces very acceptable and reproducible records. As the paper is held in place over the area, water secreted from the sweat pores places the starch and iodine in solution thereby producing definite blue-black spots on the test paper. The size of the spots is a qualitative indication of the amount of secretion from each active sweat pore, *i.e.*, the larger the spot, the greater the amount of sweat secretion from that particular sweat pore. If no sweating is present, no spot will appear on the test paper. If sweating is marked, it may be necessary to repaint the area after several such records have been made. Although these records have not proved reliably permanent for long periods of time, they produce clear and sharp points for immediate analysis, and remain clear for a period of several weeks when stored in a dark place. Permanent records are obtained by photographing the test papers.

The number of active glands is counted under the dissection microscope, or from an enlarged or projected photograph of the test paper. (For qualitative studies on sweating, microscopic examination is not necessary, because the sweat spots are clearly discernible on visual inspection.) With this technique we are able to study the general pattern of sweating on any part of the body, count the number of active glands in any given area, study changing patterns of activity, gain information concerning the nervous control of sweating, and determine directly the effects of physiological, pathological, or neurosurgical procedures involving the sudomotor mechanism.

EXPERIMENTAL RESULTS

Figure 1 illustrates the usefulness of the procedure in checking the effectiveness of operations involving the sympathetic supply to the extremeties. In this patient, the left upper extremity was partially sympathectomized, and it was desirable to know how effectively the operation had reduced sweating. Records 2 days after the operation showed a great reduction in the number of active sweat glands, but not complete abolition of sweating. There were a few active glands on all the terminal finger pads and upon thenar and hypothenar eminences, thus indicating a few remaining intact pathways to the sweat glands *via* both the ulnar
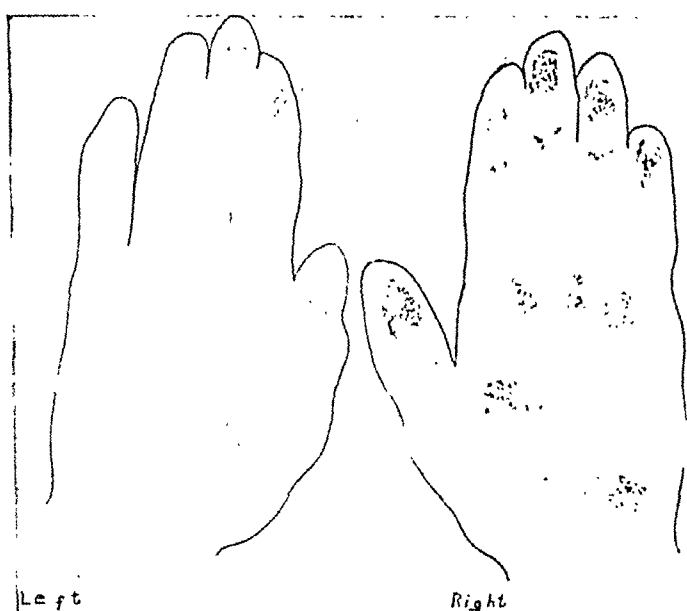
FIG. 1. SIMULTANEOUS RECORDS OF SWEATING ON THE NORMAL RIGHT HAND, AND PARTIALLY SYMPATHECTOMIZED LEFT HAND, PALMS OF A PATIENT 11 WEEKS AFTER OPERATION

Iodine was applied to the terminal finger pads, to the pads covering the metacarpelphalangeal junction, and to the thenar and hypothenar eminences, and sweating in these areas considered representative of the palmar surfaces.

and median nerves. A relatively greater number of glands on the 1st and 2 nd finger pads indicated a predominance of intact innervation *via* the median nerve. Records taken at suitable intervals thereafter confirmed this distribution (Figure 1).

Determination of maximum number of functional sweat glands

Experience has shown considerable variation in functional activity of the sweat glands in a given area. It therefore seemed desirable to establish certain reproducible conditions under which comparative studies might be made. Such studies could be accomplished under conditions of minimal, basal, or maximal activity. Of these 3 physiologically constant conditions, maximal activity proved most applicable. Since Dale and Feldberg (7) demonstrated chemical transmission of impulses to glandular cells, it has been accepted generally that the sweat glands are functionally activated through the mediation of acetyl choline. If this is true, maximal activity of functional sweat glands in a given area should be attained by introducing adequate amounts of acetyl choline into

the dermal layers of the skin. Since acetyl choline is effective for but a short time in the body, it is more satisfactory to use a drug having similar physiologic properties, but which is more stable in the body. Acetyl-beta-methylcholine (mecholyl, 0.1 per cent) [2] was used for this purpose. In order to insure relatively even distribution over a large area, the drug was introduced by iontophoresis (5 to 10 minutes at 4.5 to 5.0 milliamperes) into an area about 40 to 50 mm. square. Results obtained by this procedure were compared with results obtained by stimulation with locally applied radiant heat, hot tub bath, and with spontaneously occurring sweating in a warm room. The radiant heat was supplied by 100 watt lamp bulbs in reflectors placed 65 to 90 mm. from the test area. The heating was intense enough to produce erythema, "felt hot" to the subject, and produced a skin temperature in the area of 40 to 43° C. The hot bath experiments were carried out with the subject seated in hot water (40 to 44° C.) which came to a level just below the umbilicus. The arms were slightly elevated above the water level.

Table I shows the comparative results of such stimulations as measured on the extensor surface of the forearm. In all instances the number of glands active following mecholyl (Figure 2) and during the hot tub bath, show close correspondence, and presumably represent maximal activity in this area. The number of glands activated by

[2] Mecholyl (acetyl-beta-methylcholine chloride) furnished through the courtesy of Merck and Co.

TABLE I

Maximum number of functional sweat glands (number of glands per sq. cm.) on the extensor surface of the forearm during, or immediately following, different kinds of stimulation

Subject	Spontaneous peaks in warm room (24 to 31°C)	Radiant heat (42 to 43°C)	Hot tub bath (40 to 44°C)	Mecholyl
V R	108 132	59 200	250	230+
Ra	98 93 144 100	89 230	238 224 240	252
Fo	20 100 45	220 200	212—	245
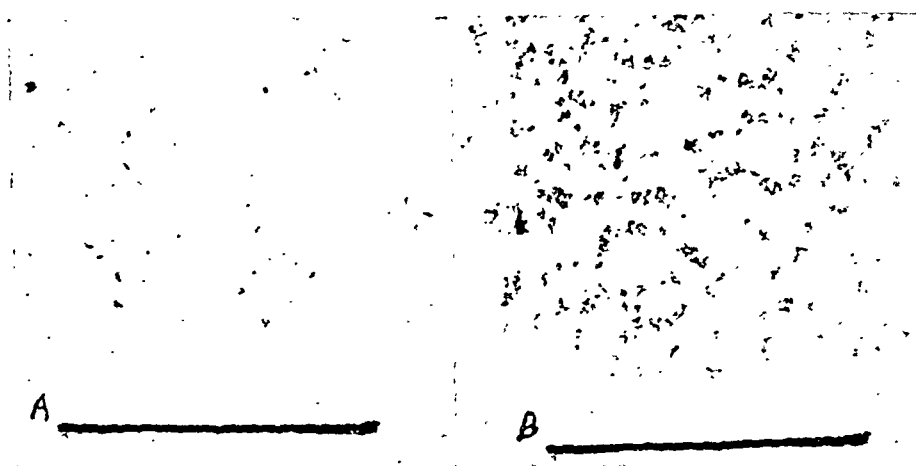
FIG. 2. RECORDS TAKEN (A) BEFORE AND (B) AFTER MECHOLYL IONTOPHORESIS ON THE SAME AREA OF THE EXTENSOR SURFACE OF THE FOREARM

The larger sweat spots in B represent increased output, and in some instances the sweat from 2 or more pores which has run together. The calibration line represents 1 cm. on the skin surface.

radiant heat depended upon the intensity of heat and extent of forearm heated, but in general this was not as effective a sudorific stimulus as the mecholyl or hot bath. This may be explained, possibly, by direct action of the drug in the one instance (mecholyl) and by the greater degree of summation, or a rise in blood temperature, in the other (hot bath experiments). The intensity of the stimulating temperature was designed to provide approximately comparable stimulating temperatures in both series of heat experiments. In all instances, the number of glands activated spontaneously in the warm room was least. These experiments have not been carried out at temperatures strictly comparable to those temperatures reached during the extreme heat of summer. Short exposures in a room temperature of 35° C. however, did not activate more glands than indicated in Table I.

From these experiments it becomes apparent that relatively extreme thermal stimulation is required to activate simultaneously all of the functional sweat glands in a given area. Thermal stimulation followed by histological examination of the same area confirms this observation, as pointed out by Kuno (5) who quotes the work of Ogata. This worker counted the appearance of sweat droplets, followed by histological sectioning of the same tissues. He described glands which seemed morphologically well developed, but which

did not appear to be functionally active under thermal stimulation. Of the "active glands," Ogata found variation from 125 to about 200 glands per sq. cm. on comparable areas. These figures compare favorably with those contained in Table I, although we are inclined to believe there are more individuals showing the higher figure. Since we find that relatively extreme environmental temperatures are required to activate as many glands as direct chemical stimulation, it seems possible that Ogata's "inactive glands" represent glands which simply did not respond to the thermal stimulation employed. Others suggest the possibility that such inactive glands may represent glands which have been functional in infancy, but which have lost functional ability in later life. It is possible that the comparatively small discrepancies may have explanation in racial and climatic factors.

It should be pointed out briefly, that maximum activity denotes more than the participation of all of the functional glands in a given area, since it may also include an increased output by the individual glands. This is demonstrated in Figure 2, in which the size of many individual spots indicates increased output of sweat per unit period of time. Experience has shown that an increase in number of glands is not necessarily accompanied by increased output per gland, and the total volume of sweat produced by a given skin area may in-

volve either or both of these functional mecha-
nisms. It is also evident from the varying size of
the sweat spots in Figure 2 that the glands do not
participate equally in output of sweat, even when
stimulated maximally.

Distribution of sweat glands

In extending studies of the maximum number of
functional sweat glands to other parts of the body,
cholinergic stimulation (mecholyl, 0.1 per cent
solution) was used in small areas as described
above. Although large individual variations be-
come apparent as one compares the number of
active glands on the same surfaces in different
subjects, there are usually similar directional
changes when one compares areas of high and low
numerical counts on different subjects.

TABLE II

*Distribution of functional sweat glands
on different body surfaces*

Area	Subject				
	Ra	Slo	Cla	She	Eng
	(functional pores per sq.cm.)				
Forearm, extensor surface	252	180	195		225
Upper arm, over biceps	220	140			170
Dorsum of hand	410	260	480	416	320
Trunk					
anterior chest	175	93			184
scapular region of back		28	44	29	17
Leg (over gastrocnemius)	116	85		63	130
Thenar eminence	368	264	440	146	200
Face					
forehead	212	37(?)			122
zygomatic	14	1	7	65	20
buccal	34	9			6

Mecholyl was used in stimulating activity in all regions
except thenar eminence in Subject Ra. Question in Sub-
ject Slo indicates probable inhibition of sweating by
mecholyl iontophoresis.

Of the areas studied in Table II, the palmar sur-
faces and the dorsum of the hand are sites of
greatest concentration of sweat glands. Although
counts on the normally sweating finger pad may
show as many as 500 or more active glands per
sq. cm., stimulation with mecholyl in these areas
has not consistently produced maximum counts.
In fact, contrasting results have been observed fol-
lowing mecholyl iontophoreses, and inhibition of
sweating in this area has been demonstrated when
dilutions identical to those producing maximum
sweating in other areas is used. It is commonly

observed, on the other hand, that close to the maxi-
mum number of glands may be activated spontane-
ously, or as a result of emotional excitement.
These divergent responses to mecholyl, combined
with the lack of sweating during thermal stimu-
lation (8), emphasizes the variant characteristics
of the palmar sweat glands. Reference to Figure
3 (segment I) demonstrates the definite pattern of
distribution of sweat pores which marks the pal-
mar surfaces, and particularly the terminal finger
pads. The sweat pores are small and somewhat
irregularly spaced along the epidermal ridges. Al-
though no pores have been observed between the
ridges, sweat sometimes spills over into the shal-
low depressions and thereby produce elongated
prints between rows of spots. Sweat output by
individual glands on the palmar surface is rela-
tively large, hence the exposure in Figure 3 (seg-
ment Hand I) was reduced to prevent coalescence
of the sweat spots.

Whereas the hypothenar eminence shows
marked ridges and definite rows of sweat spots on
the test papers, the thenar eminence is less defi-
nitely ridged and marked by folds and creases
which interrupt the spacing of the sweat pores
(Figure 3, segment H). The pores are less
densely concentrated than on the finger pads, but
are still numerous when compared with non-pal-
mar surfaces.

The dorsum of the hand is marked by a sur-
prisingly large number of sweat pores per sq. cm.,
particularly when compared with forearm sur-
faces. The output per gland per unit of time on
the dorsum appears to be significantly less than
on forearm or upper arm surfaces, as indicated by
simultaneous records taken from points 3 cm.
below (segment C) and 3 cm. above (segment B)
the wrist. Thus, although the sweat pores are
considerably more numerous on the dorsum of the
hand than on other surfaces of the arm, the total
output of sweat per unit area may not be signifi-
cantly different.

Measurements on the trunk were made from
the anterior wall of the chest and from the back
over the scapular region. In all subjects many
more functional glands were observed on the chest
wall, with a pattern of distribution quite similar
to that observed on the upper arm. Considering
the large surface area represented by the trunk
and extremities, the number of glands and output
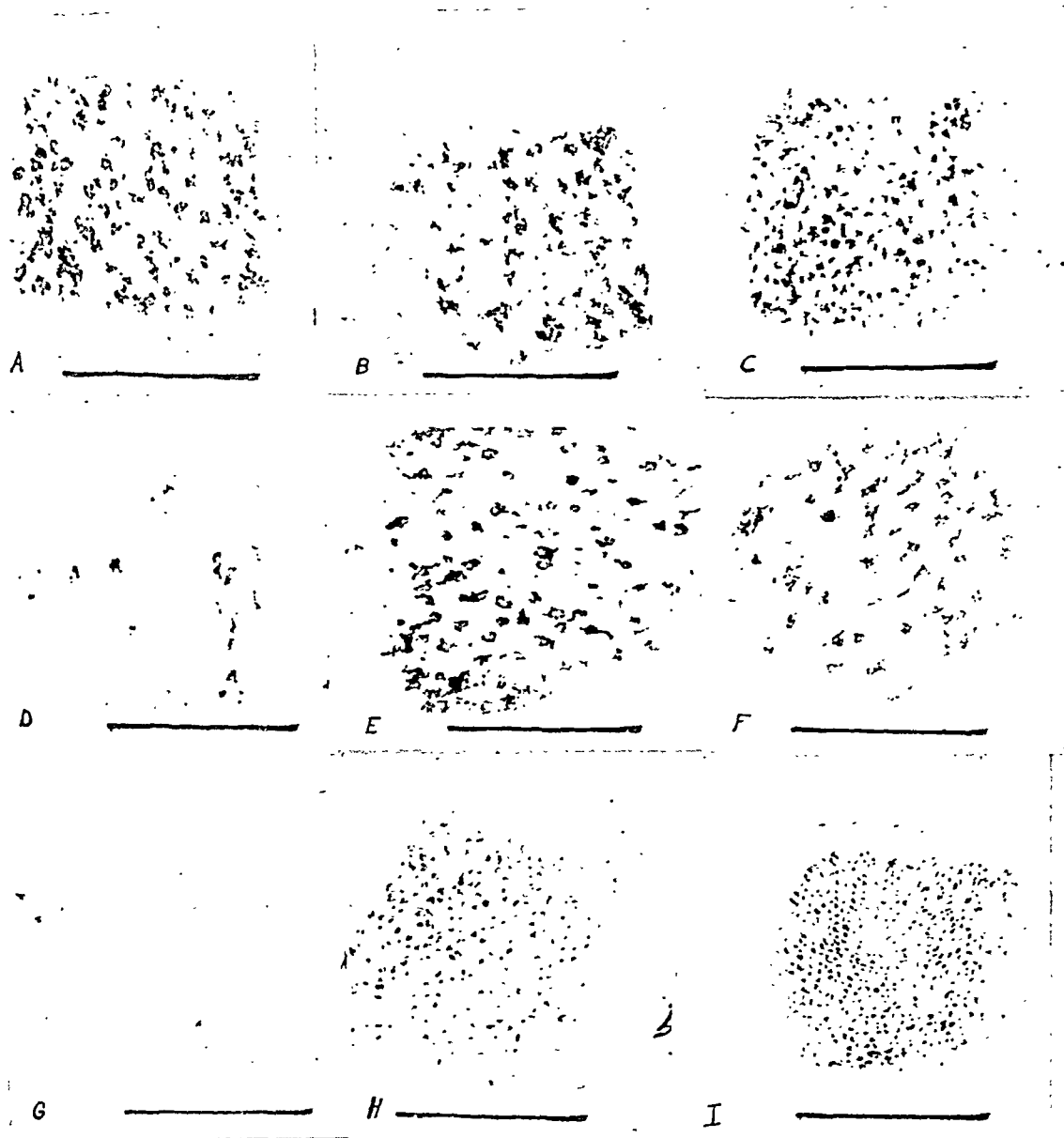
FIG. 3. RECORDS OF MAXIMUM NUMBER OF FUNCTIONAL SWEAT GLANDS FOLLOWING MECHOLYL IONTOPHORESIS (EXCEPT IN SEGMENT I, WHERE NO DRUG WAS USED) TO DEMONSTATE PATTERNS OF DISTRIBUTION ON DIFFERENT BODY SURFACES

Calibration line equals 1 cm. A. Medial surface of upper arm, over biceps. B. Extensor surface of forearm. C. Dorsum of hand. D. Scapular region of back. E. Anterior chest wall. F. Medial surface of leg over gastrocnemius. G. Buccal region of the face. H. Thenar eminence. I. Finger pad.

per gland hold particular significance in regulation of evaporative heat loss.

Cholinergic stimulation applied to different areas of the face revealed striking differences in the concentration of sweat glands. Administration of mecholyl into the skin of the zygomatic and buccal regions induced intense erythema and a local rise in skin temperature, but marked by very few functional sweat glands. This finding is in sharp contrast with temperature responses on areas in which mecholyl elicits a large blood flow accompanied by profuse sweating. Skin tempera-

ture in such experiments does not increase markedly, and indeed, more often actually falls. It is a common experience, of course, in profuse sweating over the forehead region, to note the formation of large droplets which coalesce and run down over the zygomatic and buccal surfaces. In such situations, a high surface temperature in these areas might serve the useful purpose of increased evaporation, in spite of minimal local sweat output.

Somewhat inconstant results have been obtained following mecholyl iontophoresis on the forehead, as already described for the palmar surfaces. That is, occasionally we observe an actual depression of sweating following treatment. Such inconsistencies suggest the possibility of varying sweating responses of different areas to varying concentrations of the drug, or the possibility of functional differences in the innervation of these areas.

COMMENT

The method described has been used with considerable success in determining the extent of denervation in surgical removal of the sympathetic supply to the skin, and because of its simplicity has proved particularly adaptable to the hospital room. Simplicity of procedure is not attained at expense of precise and accurate detection however, as we have demonstrated many times. In 1 patient, over 200 sweat glands per sq. cm. were found to be functional as a result of mecholyl iontophoresis on the normal forearm, while only 125 glands were functional upon a similar area of the opposite arm 1 day after removal of a portion of the sympathetic supply. While the maximum number of functional glands remained constant on the normal arm, only 26 glands were evident 30 days after the operation, and only 6 remained functional 60 days after the operation. Evidently very few fibers supplying this area remained intact. Although degenerative processes may have been complete in the 2 or 3 weeks usually allowed, further loss of function continued for several weeks, probably as a result of fibrosis-producing blocks in the remaining intact pathways during the healing of the wound.

Similarly, precise detection of very slight, yet definite, sweating shown on the distal finger pads of the denervated hand in Figure 1 indicates the functional presence of relatively few intact fibers in the ulnar and median nerves, when compared with the 400 to 500 functional glands per sq. cm. on the normal finger pads. Such precision in detection of intact nervous pathways has particular clinical significance in following the effects of peripheral sympathectomies, and in the important regenerative processes which follow.

The method has also been used in animal experiments involving monkeys, rats (9) and other laboratory animals. The animals need not be anesthetized, and only temporarily immobilized, therefore making information available under more nearly normal conditions.

SUMMARY

1. A method is described for the detection of sweating, and for enumeration of functional sweat glands in a given area under various kinds of stimulation. The method furnishes a quantitative measure of the number of active glands, and a qualitative measure of the amount of sweat secreted from each functional sweat pore. Its usefulness in checking the extent of denervation of sweating areas following sympathectomy is illustrated.

2. A comparison of thermal and direct cholinergic stimulation demonstrates that relatively severe thermal stimulation is required to activate as many glands as acetyl-beta-methylcholine (mecholyl). From theoretical considerations, the latter might be expected to activate the maximal number of functional glands in a given area in a given individual.

3. Maximum sweating consists of a combination of both increased numbers of participating sweat glands and increased output per gland, occurring in that order, with a demonstrable time interval separating the 2 levels of sweat output.

4. Patterns of distribution of sweat glands under maximum, or nearly maximum, stimulation indicate considerable numerical variation in sweat glands from area to area, and from individual to individual.

5. There is further evidence that sweat output per individual gland in different areas, and under different conditions, shows large variations.

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GALACTOSE DISAPPEARANCE FROM THE BLOOD STREAM. CALCULATION OF A GALACTOSE REMOVAL CONSTANT AND ITS APPLICATION AS A TEST FOR LIVER FUNCTION

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The use of galactose for testing the glycogenic function of the liver was suggested by Bauer in 1906 (1). Galactose is suitable for this purpose since its utilization depends upon the functional integrity of the liver, since it is non-toxic, and since its concentration can be determined readily in blood and urine (2 to 7).

Bauer's test measured the urinary excretion of galactose during the 5-hour period following ingestion of the sugar. An excessive excretion of galactose was interpreted to indicate liver damage. However, there are serious disadvantages to this procedure: the method lacks sensitivity, and it is subject to errors introduced by changes in gastro-intestinal and renal functions.

To overcome the disadvantages of an oral test Jankelson, Segal and Aisner (8) introduced an intravenous galactose tolerance test. Their technique was modified by King and Aitkin (9) and by Bassett, Althausen and Coltrin (10). These studies defined a range of normal and abnormal values for blood galactose at a given time after intravenous injection of the substance. For example, Bassett, Althausen, and Coltrin found that in normal adults no galactose remained in the blood stream 75 minutes after the injection of 0.5 gram galactose per kgm. body weight. Appreciable concentrations of galactose were still present at this time in patients with liver damage. Because of these findings, the value at 60 or 75 minutes was proposed as a diagnostic index for the presence or absence of liver damage. However, interpretation of the significance of a single value presents certain drawbacks. The blood galactose concentration at a given moment depends not only upon the function of the liver, but also upon blood and tissue space volumes. The value for galactose concentration at 60 or 75 minutes after intravenous injec-

tion represents the result of removal of galactose from the blood stream, but does not measure the velocity of the process.

In the present study, repeated determinations of blood galactose were made during a period of 1½ hours after intravenous injection of the substance. A constant expressing the rate of disappearance of galactose from the blood stream was derived from the data. Comparison was made of the values of this constant in normal subjects, in patients without evidence of liver damage, and in patients with various liver diseases. The value of this constant was also compared with the results of other liver function tests.

METHODS

A 50 per cent galactose solution [1] was prepared according to the method of King and Aitken (9). After testing the solution for sterility, it was stored at 37° C. in an incubator to prevent crystallization. The galactose test was performed usually about 2 hours after the patient had received breakfast. First, blood was drawn to furnish a blank for the non-galactose, non-fermenting copper reducing substances. Directly thereafter galactose solution corresponding to 0.5 gram per kgm. was injected intravenously in 2 to 3 minutes. Venous blood samples for galactose determination were drawn from the opposite arm at 15-minute intervals over a period of 1½ hours. Urine was collected as voided over a 4-hour period. The determination of blood galactose was based upon Benedict's method for blood sugar (11), after removal of glucose by fermentation according to Raymond and Blanco's method (12). The procedure was as follows: To 2 ml. of whole oxalated blood were added 12 ml. of 20 per cent suspension of fresh baker's yeast (washed 7 times). Fermentation was complete in 10 minutes. To the mixture were then added 6 ml. of a protein-precipitating mixture containing equal amounts of 10 per cent sodium tungstate and 2/3 N sulfuric acid. The remainder

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[1] Two brands were used, galactose, C. P. Amend Drug and Chemical Co., New York City, and galactose, Merck and Co., Rahway, N. J.

of the procedure, *i.e.*, heating, cooling, colorimetry, follows Benedict's method. However, galactose standards (10 or 20 mgm. per cent concentrations) were employed instead of glucose standards. The determination of galactose in the urine was done by Benedict's quantitative method for urinary glucose. A conversion factor of 1.14 was used.

Derivation of an expression for the rate of galactose removal from the blood

Figure 1 shows values obtained on a representative case of passive congestion of the liver. When the successive values for blood galactose are plotted against time, a smooth curve is obtained. The shape of the curve indicates that the rate of removal of galactose is not constant, but decreases as the concentration of the galactose in the blood
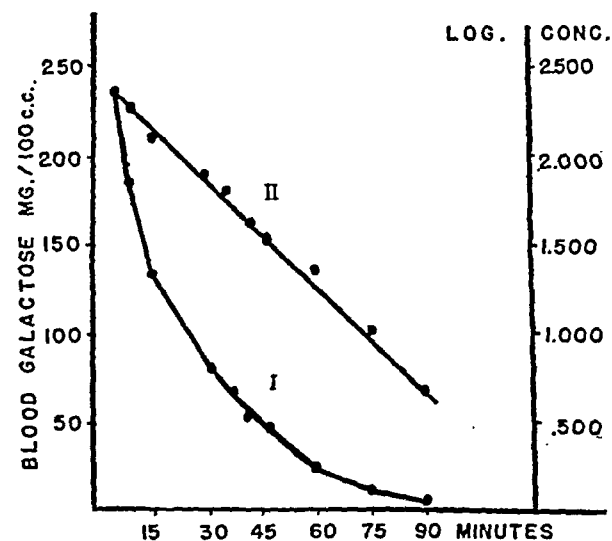
FIG. 1. CURVES SHOWING THE DISAPPEARANCE OF GALACTOSE FROM THE BLOOD AFTER I.V. INJECTION OF 0.5 GRAM OF GALACTOSE PER KGM. IN PATIENT M. W.

Diagnosis, chronic passive congestion of the liver due to heart failure. In curve I the blood galactose concentrations, in mgm. per 100 ml., are plotted against time in minutes. In curve II the logarithms of these concentrations are plotted against time.

decreases. However, when the logarithm of blood concentration is plotted against time, a straight line is obtained. This straight line may be interpreted to indicate that the rate of removal of galactose from the blood is directly proportional to the concentration in the blood. This can be expressed mathematically as:

$$-\frac{dC}{dt} = KC$$

In this equation "C" represents the concentration of galactose in the blood at time "t" and "K" represents the proportionality constant. The value of K may be obtained from the slope of the line in Figure 1, or may be calculated from the equation obtained by integration of the above expression.

Integration leads to this equation:

$$K = \frac{2.3 \ (\log C_1 - \log C_2)}{t_2 - t_1}$$

C_1 and C_2 are the concentrations at the times, t_1 and t_2. If t is expresed in minutes, K represents the fraction of the total amount of galactose present at any given time that is removed from the blood stream each minute. For convenience, the decimal fraction obtained from this equation is *multiplied by 100 so that the numerical value expresses the percentage of the amount present that is removed each minute*. This might be termed "The Galactose Removal Constant" or the G.R.C.

Although in this study 6 or 7 blood specimens were obtained from each patient at 15-minute intervals in order to establish the validity of this constant, it was found that only 2 values are required for its calculation. The galactose removal constant may be obtained with sufficient accuracy for clinical purposes from blood concentrations at 15 and 45 minutes after completion of the intravenous injection. Under these conditions the equation may be simplified to read as follows: G.R.C. = 7.6 (log concentration at 15 minutes — log concentration at 45 minutes).

Galactose Removal Constant in various conditions

Tests were performed on 64 patients.[2] The case material included 6 groups: (1) Control group of normal adults (24 to 40 years) and patients with no clinical evidence of liver disease. (2) Patients with decompensated cirrhosis of the liver, *i.e.*, patients with ascites or jaundice. (3) Patients with cirrhosis of the liver without signs of decompensation. (4) Patients with chronic passive congestion of the liver. (5) Patients with acute hepatitis. (6) Miscellaneous group of patients with liver diseases due to other causes. The grouping of patients was made mainly on the basis of clinical findings. Admittedly, one cannot be entirely certain of the diagnosis. For example, patient M. C.

obtained from patients with liver damage are more gradual, indicating a slower disappearance of galactose from the blood stream (Figure 2). Table I shows that the value for the G.R.C. varies from 4.2 to 9.5 in the controls, and is below 4 in patients with parenchymal liver damage.

Figure 3 shows the distribution of values for the G.R.C. in the several groups of patients studied. In 6 normal subjects, and in 4 hospital controls without apparent liver disease, the value was above 4. In 19 patients with decompensated Laennec's cirrhosis, and in 7 cases of "compensated" Laennec's cirrhosis, the G.R.C. was below 4. A similar low value was seen in 10 cases of chronic passive congestion of the liver due to heart failure. In 7 cases of acute hepatitis the G.R.C. was also found to be under 4.

The last column includes a miscellaneous group of patients with various diseases presumably involving the liver, some with and others without functional impairment.

There are 4 patients who showed subnormal values of the G.R.C.:

Patient T, a chronic alcoholic, who was admitted because of jaundice, showed a fatty liver by puncture biopsy, and abnormal values for other liver function tests as shown in Table I. Three years prior to this admission he presented massive ascites requiring many paracenteses.

Patient R was admitted because of severe malnutrition following resection of carcinoma of ampulla of Vater, and

FIG. 2. COMPARISON OF GALACTOSE REMOVAL FROM THE BLOOD IN A NORMAL ADULT (H. C.) AND A PATIENT WITH DECOMPENSATED LAENNEC'S CIRRHOSIS (P. B.) AFTER I.V. INJECTION OF 0.5 GRAM PER KGM.
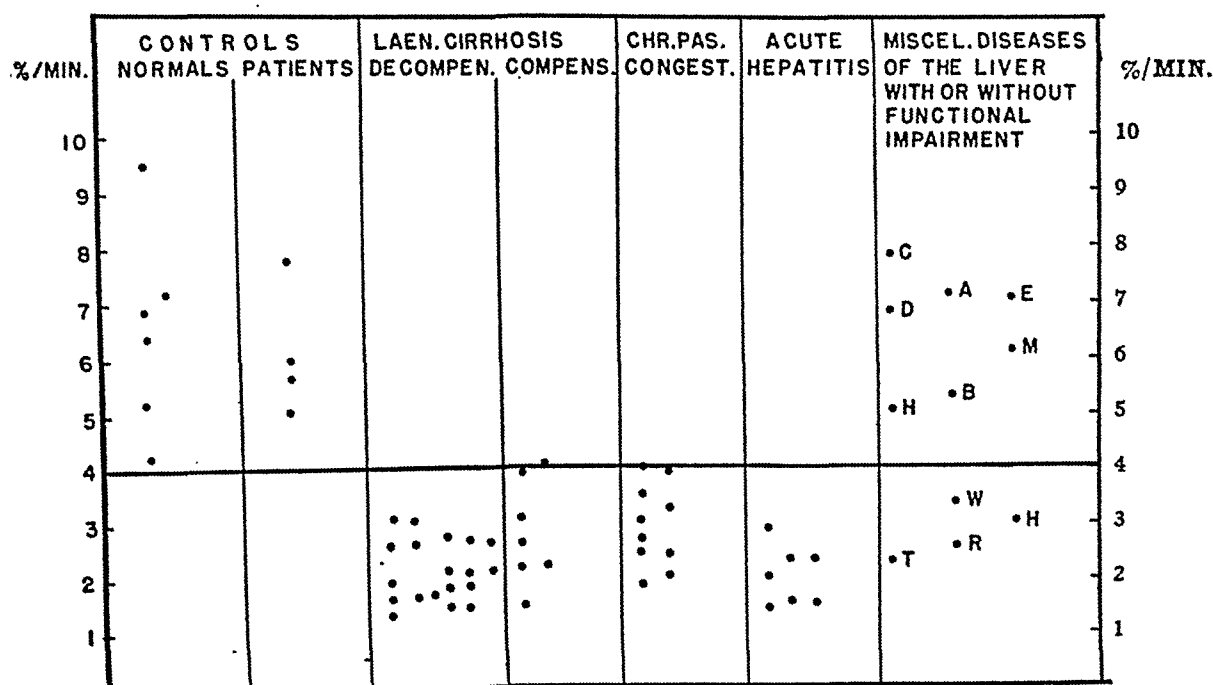
FIG. 3. THE DOTS REPRESENT THE VALUES OF GALACTOSE REMOVAL CONSTANT FOUND FOR DIFFERENT INDIVIDUALS

The letters in the last column represent the initials of the patient's last name. (See Table I.)
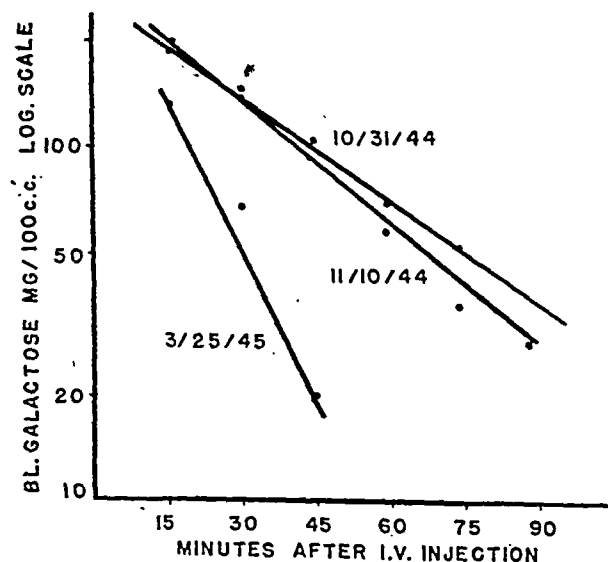
FIG. 4. GALACTOSE REMOVAL FROM THE BLOOD IN A CASE OF ACUTE HEPATITIS WITH RECOVERY

(Patient E. S.):

(10/31/44)   Second day of jaundice (Serum bilirubin 20 mgm. per 100 ml.)   G.R.C. = 1.90

(11/10/44)   Jaundice had subsided (Serum bilirubin 3 mgm. per 100 ml.)   G.R.C. = 1.94

(3/25/45)   Three months after clinical recovery   G.R.C. = 6.2

because of repeated hemorrhages due to diverticulosis of the duodenum and the large bowel. Autopsy revealed a fatty liver with slight periportal fibrosis.

Patient H, suffering from chronic glomerulonephritis, has a history of alcoholism. Hepatomegaly and laboratory signs of liver damage were found 7 years ago. These signs are no longer present.

Patient W, who is a heavy imbiber of alcohol, entered because of a mild diabetes mellitus. He shows no abnormal liver function tests other than the galactose test.

The G.R.C. was normal (above 4) in 5 patients who presented diseases of the liver that are usually not associated with functional impairment:

Patient C had "Essential Bilirubinemia," diagnosed 12 years ago. Repeated studies revealed no evidence of liver damage.

Patient D had a mild form of acute hepatitis 3 months prior to the present study. All tests were within normal limits.

Patient H had obstructive jaundice of 6 weeks' duration.

Patient M had marked hepatomegaly due to metastatic carcinoma.

Patient E had amyloid disease of liver, kidneys, and spleen.

Two additional cases with normal values of the G.R.C. were included in this group:

Patient A, a chronic alcoholic with diabetes mellitus, was treated 5 years ago for polyneuritis. At that time

there was also laboratory evidence of liver damage. At present the only abnormal liver function is bromsulfalein dye retention of 23 per cent at 30 minutes. Liver biopsy shows a normal liver.

Patient B, suffering from chronic exfoliative dermatitis with hypoalbuminemia, shows no evidence of functional impairment of the liver.

Urinary excretion of galactose in different individuals varied between 1.08 and 6 grams during the 4-hour period following the rapid intravenous injection. The greater part was excreted during the first hour. It has not been feasible to study in detail the rate of excretion since this would have necessitated an indwelling catheter. There was no correlation between the urinary excretion of galactose and the clinical status of the patients.

Comparison of Galactose Removal Constant with results of other liver function tests

A series of determinations pertaining to liver functions were performed simultaneously with the galactose test on all subjects. These included serum protein partitions, serum bilirubin, plasma prothrombin, cephalin-cholesterol flocculation, bromsulfalein retention, and urinary urobilinogen excretion.

The bromsulfalein dye test was done according to the procedure of Rosenthal and White (13) employing 5 mgm. dye per kgm., and taking the end point at 30 minutes. Determination of the concentration of the dye in the serum was made in the Klett colorimeter with filter no. 54. Retention of over 10 per cent of dye was considered to be abnormal. Serum proteins were determined by the micro-Kjeldahl method; serum bilirubin, by the method of Evelyn and Malloy (14); cephalin-cholesterol, by the method of Hanger (15); and prothrombin, after the method of Quick (16), using "Stypven" (Burroughs-Wellcome) as the thromboplastic agent. Normal values for prothrombin time ranged from 14 to 18 seconds by this method. Urobilinogen was determined by the simplified method of Watson (17). Excretion of more than 0.5 mgm. per hour was considered to be abnormal.

The results are shown in columns 11 to 18, Table I. [1]There was a good correlation between the values of G.R.C. and other liver function tests. In all instances where parenchymal liver damage was indicated by the other tests, the values of the G.R.C. were below 4.[1]

The galactose test seems to be as sensitive as the bromsulfalein dye retention test for the detection of liver damage, and it has the added advan-

take of being feasible in the presence of jaundice. Technically, of course, it is more difficult. In the absence of jaundice these 2 tests can be performed simultaneously.

Both the bromsulfalein test and the G.R.C. show persistent abnormality in Laennec's cirrhosis, even though the patient may show signs of considerable clinical improvement. In other words, there may be loss of ascites and jaundice, gain in strength and return to normal activity without comparable improvement in these tests. The significance of this finding is not clear. It is possible that these functional changes result from structural changes in the vascular bed of the liver.

DISCUSSION

The Galactose Removal Constant gives an over-all measure of the disappearance of this substance from the blood stream. This disappearance involves several mechanisms, namely, diffusion into the extracellular fluid compartment of the body, utilization of galactose, and excretion in the urine. In a detailed experiment on 5 dogs, Dominguez and Pomerene (18) analyzed these separate aspects of galactose metabolism. After the rapid intravenous injection of various amounts of galactose, they determined the values for this substance in the blood and urine at frequent intervals over a period of 6 to 7 hours. Their data show that the rate of excretion is proportional to the plasma concentration, and is independent of the amount injected . Constants were also derived for the rate of utilization. Whereas their work provides precise information, the procedure and the formulae are somewhat too elaborate for clinical application.

In the present study it is assumed that a state of equilibrium has been reached between galactose in the blood stream and the galactose in the tissue spaces within the first 15 minutes of injection, and that the disappearance curve thereafter gives an index of galactose utilization. The rate of excretion is not taken into account, since under the present conditions only about 10 per cent of the injected galactose is recovered from the urine. Moreover, since there appears to be little difference between normals and patients with liver damage in the urinary excretion of galactose over a 4-hour period, the neglect of this factor would seem to be of little practical consquence.

SUMMARY AND CONCLUSIONS

1. The rate of disappearance of galactose from the blood stream after intravenous injection of 0.5 mgm. per kgm. was studied in normal adults, patients with Laennec's cirrhosis of the liver, chronic passive congestion of the liver, hepatitis, and other affections of the liver with and without functional impairment.

2. It is suggested that *the per cent of galactose removed per minute be termed the Galactose Removal Constant.*

3. A simplified formula is given by which the Galactose Removal Constant can be calculated from 2 blood specimens taken 15 and 45 minutes after intravenous injection of galactose.

4. The Galactose Removal Constant varied between 4.2 and 9.5 in the control group, whereas it was below 4 in patients with impaired liver functions.

5. There was a good correlation between the changes in the Galactose Removal Constant and tests pertaining to other liver functions.

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DETERMINATION OF THE RATE OF DISAPPEARANCE OF MUSTARD GAS AND MUSTARD INTERMEDIATES IN CORNEAL TISSUE [1]

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Prior to World War II, the time of persistence of mustard gas (bis β-chloroethyl) sulfide (hereafter referred to as H), in tissues had not adequately been determined. Although it was known that in aqueous environments H is rapidly hydrolysed to innocuous products, it seemed possible that this substance, because of its high lipoid solubility, might persist for much longer periods in the lipoid fractions of tissues. This possibility required investigation because of its bearing upon the potential efficacy of methods of decontamination, or treatment based upon the introduction into tissues of substances capable of reacting with and detoxifying H. The results of numerous investigations carried out during the war years have established that H, as such, rapidly disappears from the skin and eye. The present paper, based on work carried out in 1942, presents the results of one of the quantitative chemical investigations bearing on this point. Similar conclusions may be derived from the results of other work, notably that of Henriques, Mortiz, Breyfogle and Patterson (1941 to 1944) (1) on the skin, and that of Snell (1942, 1943) (2) on the cornea.

It is the purpose of this paper to report the results of an investigation designed to measure the rate of disappearance of H in tissue (cornea), and to correlate the quantity of H reacting with the degree of one of the effects produced by H on corneal tissue, viz., inhibition of turgescence which results normally when corneas are immersed in water. The latter effect was chosen for quantitating the effect, since previous studies by Cogan and the authors had shown that exposure to H gave rise to reproducible changes in the degree of swelling.

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[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

METHODS

Trephine buttons (70 mgm.) were cut from beef corneas and immersed for 10 minutes in liquid H which was kept at a temperature of 12° C. to minimize the chemical reaction of H with the cornea during the immersion period. The corneal buttons were then quickly rinsed in 3 washes of purified kerosene (hereafter called PK), and 1 wash of petroleum ether. Following this they were either extracted immediately for 2 hours in a mixture of 80 per cent cyclohexane and 20 per cent PK at 0° C., or else were placed in empty stoppered bottles for varying lengths of time, and maintained at 23° C. or 37° C. to permit the H to react before extraction of any unchanged H.

Analyses of these extracts for H were performed by the method of Kinsey and Grant (3).

Following the extraction period, the corneal buttons were allowed to swell in distilled water for 18 hours and were then weighed. The amount of swelling was calculated on the basis of 100 per cent as the initial weight. Cyclohexane and PK used in the extraction process were found to have no effect on subsequent corneal swelling in water. Fifty control corneal buttons swelled an average of 570 per cent.

RESULTS

The curves of Figure 1A show the amount of unreacted H remaining in the corneal pieces after standing for various lengths of time at 23° C. and 37° C. following the 10 minute exposure period. It will be seen that half of the H no longer can be recovered after 13 and 3 minutes for these temperatures, respectively. These half-lives correspond almost exactly to those found for H in 0.60 per cent NaCl solution at the same temperatures. There was considerable variation in the amount of recoverable H in individual corneal pieces, which necessitated the use of relatively large number of pieces for each point composing the curves.

Figure 1B shows the amount of turgescence observed on these same corneal pieces. It is apparent from the figure that the observed effect on the cornea, as measured by the degree of inhibition
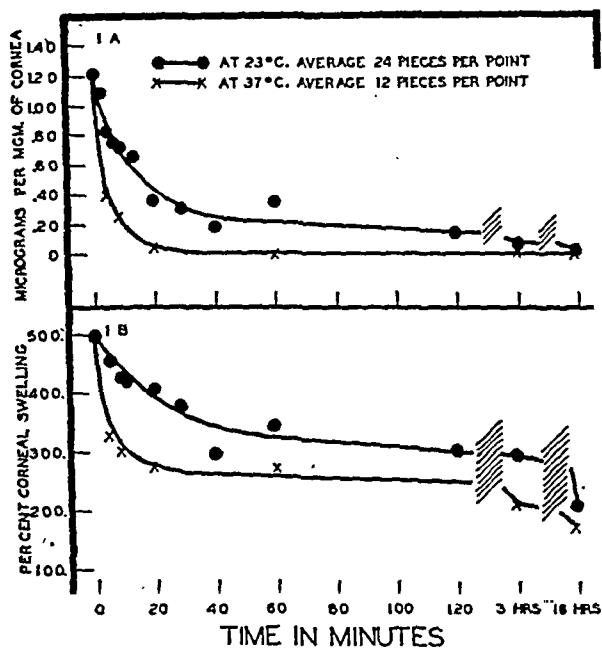
FIG. 1A. The Amount of Unreacted H Remaining in Corneal Pieces After Standing for Various Lengths of Time at 23° C. or 37° C.

FIG. 1B. The Amount of Turgescence Found in Corneal Pieces After Standing Various Lengths of Time Following a 10-Minute Exposure to H

of turgescence, tends to parallel the disappearance of the H at the 2 temperatures. This relationship of effect produced for a given amount of H reacted, at the 2 temperatures investigated, may be more clearly seen from Figure 2. There the amount of H left in the cornea is plotted against effect, expressed as per cent inhibition of turgescence, where

100 per cent inhibition represents absence of swelling capacity, and 0 per cent inhibition represents swelling of normal unexposed corneas (570 per cent). It will be seen from the line composed of filled circles, which shows the data from the 23° C. experiments, that the effect up to approximately 1.4 γ of H per mgm. of cornea, varies directly with the amount of H which has reacted. Above this amount it is evident that the effect no longer is directly proportional to the amount of H reacted, but increases faster than H disappears. Moreover, the effect from a given amount of reacted H appears to be somewhat greater at 37° C. (crosses,
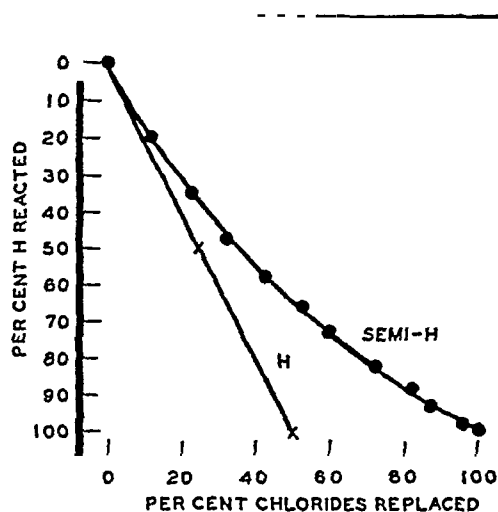
FIG. 3. The Relation Between the Percentage of H Reacting and the Percentage of Available Chlorides in the H or Semi-H Which Have Been Replaced

Figure 3) than at room temperature. Both the changing slope of the 23° C. line, and the apparently more uniform effect produced at 37° C. are explicable on the assumption that 1 or more additional substances are formed which also contribute to the inhibition of turgescence. From studies being pursued simultaneously, it was found that chloroethyl-hydroxy-ethyl sulfide, hereafter referred to as Semi-H, is produced by the degradation of H in aqueous solution. It seemed probable that the presence of Semi-H, which was not determined in H analyses, might account for the linearity of the 23° C. curve, as well as the greater observed effect per unit of reacted H at 37° C. To test this hypothesis it seemed necessary to establish whether the effect produced in the corneal

FIG. 2. The Relation Between the Effect Produced on Inhibition of Corneal Turgescence and the Quantity of H Which Had Reacted Within the Cornea

tissue more closely paralleled the replacement of both chlorides of H. It is apparent that if this idea were substantiated it would be of practical importance to know the overall rate of reaction of H as well as its reactive products.

The rate of replacement of the first chloride is given directly by the measurement of the rate of disappearance of H. An approximation of the rate of replacement of the second chloride can be obtained from the rate of breakdown of the chief analogue of H in which 1 chloride has been replaced, i.e., Semi-H.

The rate of breakdown of this compound was determined experimentally in 0.60 per cent saline and compared with that for H. From these data the proportion of both the first and second chlorides which would be replaced when any given fraction of H had hydrolyzed, was calculated from the equation representing the consecutive reactions which occur when H hydrolyzes in 0.60 per cent saline solution. This was confirmed experimentally using pure Semi-H, and the temperature coefficients for the rate of replacement of the first and second chlorides were also found to be essentially the same. It follows that the total amount of replaceable chloride which has been available for any given fraction of H hydrolyzed is independent of temperature.

Hence, the data from Figure 3 may be used to determine the total number of chlorides which have been available for replacement from both H and Semi-H.

The amounts of H as taken from Figure 2 were now expressed on a percentage basis, whereby 1.84 γ of H per mgm. of cornea represented 100 per cent of the H reacted. The proportion of the total chlorides replaced for each percentage of H reacted was read off Figure 3 (filled circles). These values were now used as the abscissa of Figure 4 and plotted against effect produced (per cent inhibition). Thus, Figure 4 shows the effect produced on the cornea as a function of the proportion of total replaceable chlorides. It will be seen that the effect is closely proportional to the number of chlorides available for replacement from both H and Semi-H. This is in contrast to the poor proportionality found when . H alone was considered to be effective (Figure 2). From the fact that this relationship

holds equally well whether the measurements were made at 23° C. (filled circles) or 37° C. (crosses), it is apparent that the relationship is independent of rate of reaction.

Since it appears from the foregoing that H hydrolysis intermediates are capable of producing the same effect as the original H on the cornea (inhibition of swelling), it is necessary to know how long these active substances may be present in the cornea. This information can be obtained from Figure 3, where it will be seen that in 0.60 per cent saline (as found in the cornea) the "overall" half-life of H and its monochloro intermediate, Semi-H, is about 1.4 times longer than the half-life of H itself. Applying this factor to the
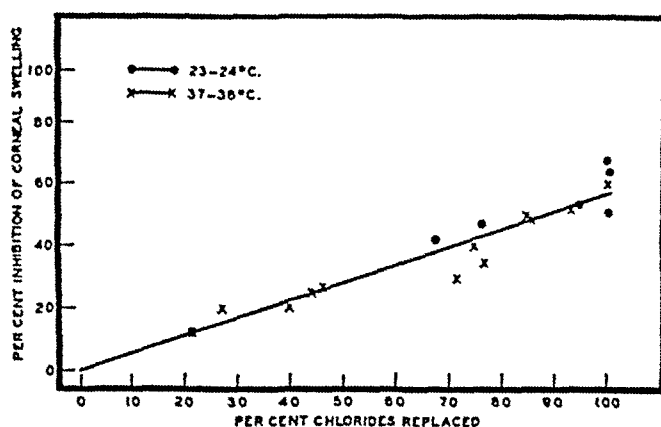
FIG. 4. THE RELATION OF THE INHIBITION OF CORNEAL TURGESCENCE AND THE AMOUNT OF H PLUS H HYDROLYSIS PRODUCTS WHICH HAVE REACTED AT 23° C. OR 37° C.

experimental measurements of the rate of H disappearance in the cornea, it is apparent that where the half-life at 23° C. was 13 minutes, the overall half-life was 18½ minutes. At 37° C. the half-life of H was 3 minutes, and the overall half-life was 4.2 minutes. It will be recalled that the "overall" half-life also gives an expression of the time required for production of half the observed inhibition of corneal swelling.

DISCUSSION

Although corneal turgescence was used as a convenient measure to quantitate the effect produced, the dose of H required to produce a significant effect on inhibition is far greater than that which gives rise to severe ocular lesions. It should

not be implied, therefore, that inhibition of corneal turgescence is to be considered as a primary sign of H poisoning in the eye.

These experiments indicate that H and its labile hydrolysis intermediate, presumably Semi-H, exist as such, in corneal tissue for a remarkably short time at body temperature, and that the reaction which occurs during this period results in at least one effect on the tissue. No evidence was found to indicate that the hydrolysis rate in the cornea is essentially different from that in pure aqueous solutions. Thus, in this tissue, at least, no preservation of H in the lipoid phase was evident.

In view of the frequent attempts to institute therapy against H poisoning by introducing substances into tissue which are designed to react with H or its toxic degradation products, it is important to point out that the results of the present investigation would indicate that such treatment would have to be carried out within less than 3 to 5 minutes to be of practical value.

SUMMARY

1. The rate of disappearance of H in corneas *in vitro* at 23° C. and 37° C. was determined by analysis of cyclohexane-kerosene extracts. For these 2 temperatures, the half-life of H was found to be approximately 13 and 3 minutes, and the over-all half-life of H and its monochloro hydrolysis product was $18\frac{1}{2}$ and 4 minutes, all respectively.

2. The effect on the corneal tissue was measured by subsequently determining inhibition of swelling of the corneal pieces when placed in water. It was found that the effect was proportional to the number of chlorides of both H and H hydrolysis intermediates available for replacement.

3. The findings indicate that therapeutic agents designed to react with H within tissues would be without benefit unless used within 3 to 5 minutes after contact with the vesicant.

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DEMONSTRATION OF A TOXIC FACTOR IN THE BLOOD OF RATS SHOCKED BY BURN [1]

By MYRON PRINZMETAL, H. C. BERGMAN, AND H. E. KRUGER

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In previous studies published from this laboratory (1) it was shown that 2 major factors are implicated in the production of burn shock in mice and rats; the one, fluid loss at the site of the thermal injury, and the other, stagnation of blood in atonic visceral capillaries. Capillary atony was demonstrated in burn shock whether thermal trauma was accompanied by much or little local fluid loss. In certain forms of thermal injury there is very little edema formation (2); yet the circulatory blood volume as represented by the bleeding volume is drastically reduced and the animal dies in shock (1). In this instance, local fluid loss is eliminated as the principal cause of the reduction in the bleeding volume, and the pooling of blood in the capillary bed is chiefly responsible for the decrease in the effective circulating volume. In burn shock the number and diameter of open capillaries were found to be considerably increased (1, 3), and the amount of blood retained in the viscera significantly augmented. Atony of the capillary blood vessels is a primary disturbance since it develops within 2 minutes after a severe burn. Following mild burns, capillary atony persists in surviving animals for less than 24 hours. In order to demonstrate capillary congestion, controlled observations were made upon the hemoglobin content of visceral organs after exsanguinating the animal. There was little or no difference in the apparent degree of congestion in the burned and unburned animals when sacrificed by a method other than bleeding, but a striking difference was demonstrated after exsanguination. After bleeding, the organs of a normal unburned animal became pale and bloodless, whereas in burn shock the tissues remained dark and engorged.

A toxic factor has been previously demonstrated in experiments in which injection of blood from burned rats into normal rats caused a significantly lower bleeding volume than injection of blood from normal animals (1). The studies reported in the present communication were undertaken to determine whether the perfusion of blood from a burned animal through a normal kidney would reproduce the capillary congestion which is characteristic of burn shock.

METHODS

Adult male, Long-Evans rats weighing 250 to 350 grams were anesthetized with ether. The pedicle of the right kidney in each test animal was tied and the kidney removed immediately by severing the pedicle distal to the ligature. Each animal received two ml. of heparinized blood taken from burned rats. The blood was removed from the abdominal aorta of the anesthetized donor rats 3 to 4 minutes after they were burned to the neck at 100° C. for 20 seconds, and injected into the abdominal aorta of the test rats just proximal to the origin of the renal artery. To facilitate the injecton, the needle was bent at an angle of approximately 120°. The abdominal aorta below the renal artery was momentarily compressed by the finger in order to insure the direct passage of the major portion of the injected blood through the left kidney (Figure 1). This procedure would seem to be more nearly physiologic than most standard methods of perfusion since the circulation is kept intact; the only departure from the normal being a temporary rise in the renal arterial pressure. Furthermore, a very high concentration of toxic factor would be expected to enter the perfused organ.

The left kidney in each of the test animals was removed immediately after perfusion and allowed to self-exsanguinate, and the hemoglobin content of both kidneys was determined. Thirty-eight control test rats prepared in precisely the same manner were given an injection of 2 ml. of heparinized blood from unburned animals.

The amount of hemoglobin in the kidneys was determined as follows. Hemoglobin was extracted with a buffered salt solution and measured photometrically after conversion to cyanmethemoglobin. The details are described in another publication (4). The reliability of the method was tested by making comparative estimations of like amounts of tissue taken from the same organ. These gave values within 5 per cent of one another.

In order to ascertain the number and diameter of open capillaries in the kidneys, duplicate sets of organs were
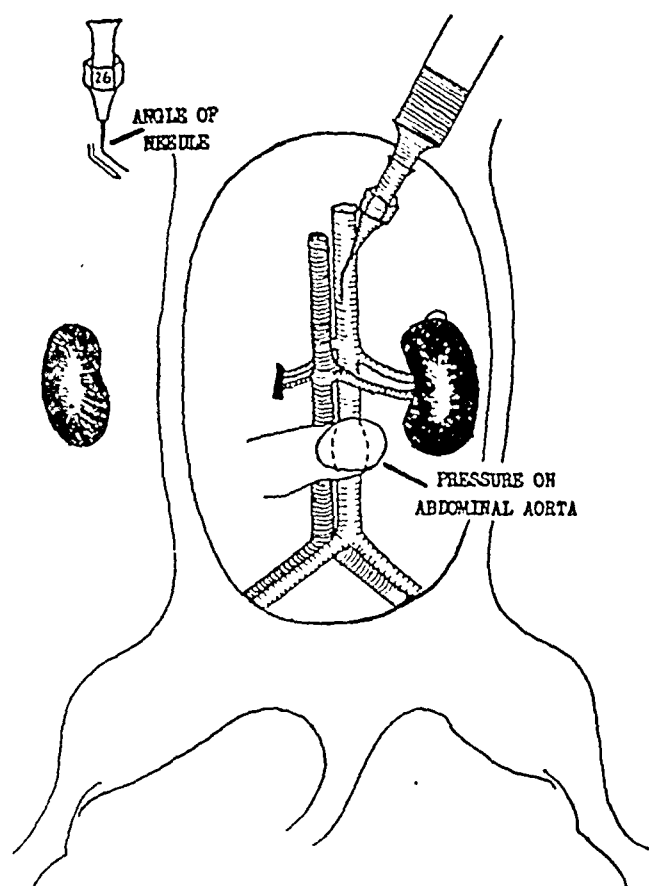
FIG. 1. ILLUSTRATION OF METHOD OF PERFUSING A
KIDNEY WITH BLOOD

prepared for histologic examination. The method of
preparation and staining of the sections was described in
a previous publication (1).

RESULTS

In 38 rats, the kidneys which were perfused with
normal blood were found to contain 1.20 ± 0.039 [2]
times as much hemoglobin as the unperfused kid-
neys. Manipulation of the kidney and the tem-
porary rise in the renal arterial pressure during
perfusion may explain the small increase in the
hemoglobin content of the perfused kidney. In
a parallel group of rats in which 1 kidney was per-
fused with blood from burned animals, the ratio of
the hemoglobin content of the perfused kidneys
to that of the unperfused kidneys was $1.54 \pm$
0.074.[2] Since comparison of these ratios gives a P
value of less than 0.001, the difference of the mean
values is statistically significant.

There is a striking difference in the gross ap-
pearance of the kidneys removed before and after
injection of blood from a burned animal. The un-

[2] Standard error of the mean.

perfused kidney self-exsanguinates rapidly and
becomes pale and wrinkled, whereas the perfused
kidney shows only minimal bleeding and presents
a dark, engorged appearance. The perfused kid-
neys resemble kidneys taken from animals in
shock. Kidneys perfused with normal blood do
not differ in appearance from unperfused normal
kidneys.

Table I shows the capillary counts in 2 animals
injected with normal blood, and in 2 animals in-
jected with blood from burned rats. In rats in-
jected with normal blood, the average counts were
10.6 open capillaries in 10 standard fields (magni-
fication 440) in the control kidney, and 8.2 open

TABLE I
*Capillary counts in kidneys perfused with blood
from normal and burned rats*

Perfusate	Capillary counts*				"P" value†
	Control kidney		Perfused kidney		
	mean	*std. error*	*mean*	*std. error*	
Normal blood	11.3	±1.4	7.8	±1.2	>0.05
	9.9	±1.8	8.6	±1.7	>0.6
Blood from burned rats	6.9	±1.8	37.9	±1.9	<0.001
	7.2	±1.3	23.9	±1.6	<0.001

* Average number of open capillaries in 10 standard
fields (magnification 440).
† "P" value of less than 0.05 indicates that the difference
of the means is statistically significant.

capillaries in the perfused kidney. The corre-
sponding values in rats injected with blood from
burned animals were 7.1 and 30.9, respectively.
The renal capillaries in animals which received
blood from burned rats were abnormally dilated,
and contained many more cells than those in ani-
mals which received normal blood (Figure 2).

DISCUSSION

In previous investigations (1) an atonic state
of the vessels of the capillary bed leading to visceral
congestion has been shown to be one of the primary
factors in the initiation of the shock syndrome in
scalded animals. Capillary congestion was espe-
cially prominent in the kidneys, liver, heart, intes-
tine, and adrenal glands of the shocked animals,
and could be demonstrated within a few minutes
after a severe burn (1). Briefly stated, the re-
sults of the experiments described in this com-
munication indicate that perfusion of a rat's kid-
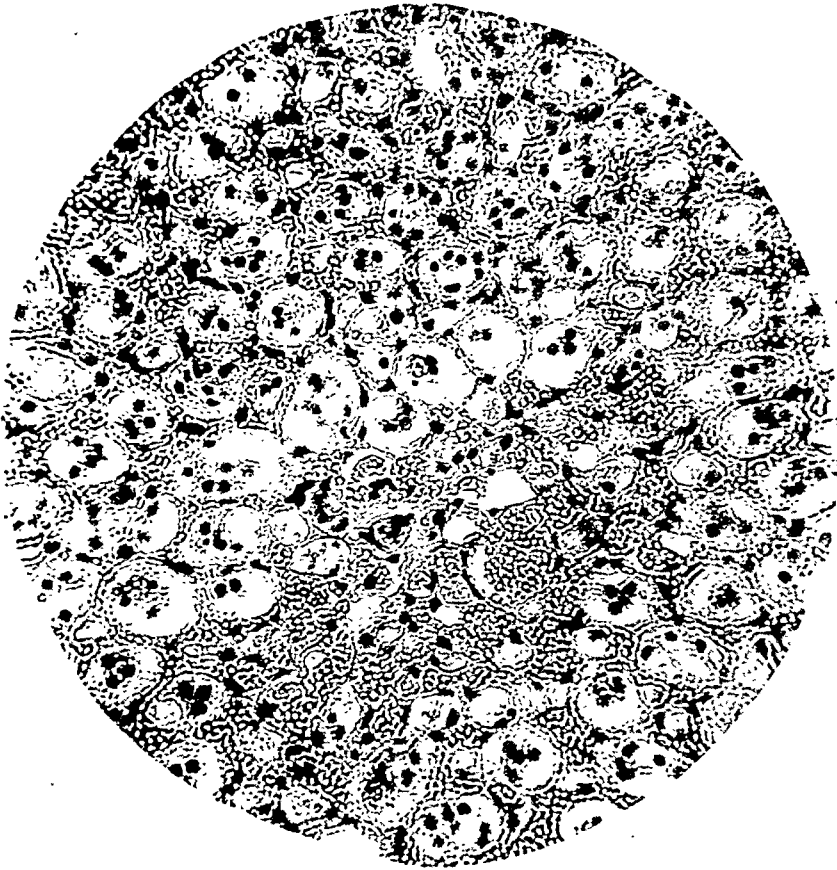
FIG. 2. PHOTOMICROGRAPH SHOWING OPEN AND DILATED CAPILLARIES IN MEDULLARY REGION OF RAT KIDNEY FOLLOWING PERFUSION WITH BLOOD FROM A BURNED RAT (magnification 440)

ney with blood from a burned animal reproduces the capillary congestion which is characteristic of burn shock. Since capillary atony can be induced in a normal rat either by a burn or by the injection of blood from a burned animal, it is reasonable to conclude that the capillary congestion is due to a toxic factor which circulates in the blood of a burned animal. It is important to emphasize that this factor is not necessarily a newly formed substance; it may represent merely a change in the concentration or in the physical state of a normal blood constituent. Of interest in this respect are the observations of Shen, Ham and Fleming, who demonstrated changes in the morphology and osmotic fragility of red blood cells after thermal burns in human beings (5). There have been recent reports on the presence of toxic substances in other forms of shock (6 to 10).

Recently, Chambers, Zweifach, and their associates (11 to 13), investigating vascular reactions in shock, described 2 phases, the second of which was characterized by a progressive decrease in the constrictor activity of metarterioles and precapillary sphincters. Sequestration of blood from the active circulation by pooling in the capillary bed would lead eventually to failure of the venous return to the heart.

Zweifach (14) showed that 2 types of blood capillaries could be distinguished according to whether muscular elements are present or absent in their walls. These are (1) the arteriolovenular capillaries ("muscular capillaries") and (2) the true capillaries ("non-muscular capillaries").

Both types of capillaries possess tone, and in the latter the presence of tone must be ascribed to the endothelial cells that comprise its wall. It cannot be denied that the capillaries respond passively to tonic changes in the metarterioles and precapillary sphincter musculature, but the participation of active changes in the tone of the capillaries themselves cannot be excluded as a factor capable of influencing their calibre. The degree of self-exsanguination of an extirpated organ in the normal and shocked animal, respectively, throws light upon the question of whether the true capillaries actively participate in the emptying of the capillary bed. If, for instance, a kidney is removed by severing the renal pedicle, the organ promptly self-exsanguinates, and it would appear highly improbable that the capillary bed could be emptied to so great an extent without active contraction of the capillaries themselves. On the other hand, a kidney removed from a shocked animal retains almost all of its blood, and the remarkable degree of dilatation and congestion of all the vessels in the capillary bed as shown in the histologic sections is not easy to explain, unless it is assumed that the true capillaries have suffered a loss of tone.

It is not at all certain that the toxic factor postulated in the present communication is identical with the vasodepressor principle described by Chambers and his associates (11 to 13), and the question of their relationship remains for future studies to determine.

SUMMARY

1. A simple physiologic method of renal perfusion is described.

2. Perfusion of the normal rat's kidney with blood taken from a burned rat reproduces the toxic capillary atony which is characteristic of burn shock.

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THE ACTION OF HISTAMINE ON THE RESPIRATORY TRACT IN NORMAL AND ASTHMATIC SUBJECTS [1]

By JOHN J. CURRY

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While investigating the systemic effects of histamine in man, Weiss and his co-workers (1, 2) observed that the intravenous injection of histamine frequently precipitated asthmatic-like attacks in patients with asthma. They also noted that it decreased the vital capacity of patients with bronchial or cardiac asthma, bronchitis or emphysema. The details of these disturbances were not reported. In view of the recent interest in antihistamine preparations, it appeared worth-while to study more thoroughly the effects of histamine on the respiratory tract in man.

METHODS

Histamine was administered to 3 groups of subjects in whom the degree of bronchoconstriction was assayed chiefly by recording the measurement of the vital capacity. The first group was comprised of 10 normal subjects. The second group included 10 patients who had a history of severe allergic disturbances, including in some a history of asthma, but who were asymptomatic at the time of observation. The third group was made up of 9 patients with varying degrees of chronic bronchitis, emphysema and asthma. An attempt was made to include in this group young, adult patients with uncomplicated continuous asthma, and to exclude patients who because of language or other difficulties could not perform the vital capacity tests without marked unaccountable variations.

Because of seasonal and other variations known to occur in asthmatic patients, one patient, B. R., a 28-year-old, single female with chronic asthma, was studied several times during the course of a 10 month period. Through training, she became an extraordinarily reliable subject, and was used for the majority of the observations. We are grateful to her for her cooperation. The other subjects were helpful mainly in confirming and enlarging the information obtained from patient B. R. Although several methods (3 to 6) have been advocated for the study of respiratory function in asthma, the determination of the vital capacity was one of the first, and is simple and fairly reliable. Since but a short period of time is required to perform the test, rapid variations in respiratory function can be detected. It was noted, however, that to do a series of vital capacity measurements in some asthmatic

patients was not an easy procedure. This was due not only to fatigue, but also to coughing spells precipitated by forced expiration, especially in patients with bronchitis. A Standard Benedict Roth Metabolism Apparatus was used for the vital capacity measurements, which were recorded on a smoked drum revolving at a speed of 34 cm. a minute with a signal magnet marking 1 second intervals. By this method the slope of the expiratory curve, as well as its volume, could be studied, and bronchoconstriction distinguished from failure to perform the test with a maximal effort. While theoretically the reduction in vital capacity could be due either to bronchospasm or to edema formation, the rapidity of the changes found in this study would indicate that bronchoconstriction was the major factor. However, further studies would be needed to determine exactly the relative importance of these 2 factors in producing a decreased vital capacity after histamine.

Subjects were brought to the laboratory at least 2 hours after a meal, and were seated comfortably. No medication had been given for at least 12 hours prior to the test, and in most instances, epinephrine had been the only medication previously prescribed. During a 20-minute rest period the subject was instructed in the nature of the test, and the possible reaction that might be encountered. Assurance was given that any severe symptoms would pass away very quickly and could be promptly relieved by epinephrine. At the end of this time, from 3 to 6 vital capacities were measured to serve as controls. A varying period of time was allowed between tests in order to avoid fatigue. In subjects being studied for the first time, a greater number of tests were occasionally made until satisfactory checks were obtained. In each experiment the entire range of the control vital capacities was recorded on the charts, even though in many instances there was a good correlation between 2, 3 or 4 determinations.

The reaction of the tracheobronchial tree to histamine was then determined by injecting gradually increasing single doses of the drug into the deltoid muscle, and repeating the measurements of vital capacity. The preparation used was histamine acid phosphate, 1 ml. of which was equivalent to 0.2 mgm. of histamine base. All doses of histamine refer to the base. Much smaller amounts were then administered intravenously. Histamine was also given under the tongue and by nebulization, using the ordinary hand-type nebulizer. In these instances a more concentrated solution was employed, in which 1 ml. of histamine acid phosphate was equivalent to 1.0 mgm. of

the base. The time of reactions and intervals between tests were measured by a stopwatch.

RESULTS AND COMMENT

In 10 normal subjects studied by this technique, no notable change in the vital capacity was observed when a dose of 0.36 mgm. of histamine was administered intramuscularly, or 0.02 to 0.03 mgm. given intravenously. Likewise, in 10 patients with a history of strong allergic tendencies, 5 of whom had a history of asthmatic attacks, no serious alteration in vital capacity was produced by similar doses of histamine. In 3 of the 5 cases with a history of asthma, a very slight reduction in vital capacity took place 30 seconds after the intravenous injection of histamine, but the change was no greater than differences observed in the control vital capacities. A study of the effect of larger doses of histamine was precluded by the severity of the side reactions.

Eight of the 9 subjects with active asthma and varying degrees of bronchitis and emphysema showed sensitivity of the tracheobronchial tree to histamine. This sensitivity varied from person to person, and in the same person varied with the degree of asthmatic symptoms (Table I). Patient P. W., a 26-year-old male with a history of wheezing and shortness of breath occurring yearly in damp weather, in whom fine wheezes could be heard throughout the lung fields at the time of the test, failed to show any notable decrease in vital capacity from a dose of 0.03 mgm. of histamine intravenously.

INTRAMUSCULAR HISTAMINE

When histamine was injected intramuscularly in sensitive asthmatic patients, a decrease in the vital capacity was observed 1 minute later, and the greatest decrease usually occurred in the second or third minutes after the injection. The vital capacity usually returned to normal within 20 to 30 minutes. Thus, in one subject, B. R., the intramuscular injection of 0.06 mgm. of histamine resulted in a drop of 1,035 ml. in the vital capacity, from the control level of 3,155 ml. to 2,120 ml. in 1 minute. At 2 minutes the vital capacity had decreased further to 1,920 ml., and 3 minutes after the injection it measured 1,955 ml. At 30 minutes the vital capacity had returned to near the control range, and measured 3,050 ml. When gradually increasing dosages, ranging from 0.02 to 0.16 mgm., of histamine were injected at intervals intramuscularly, increasing amounts of bronchoconstriction were produced (Figure 1). When gradually decreasing dosages were employed, ranging from 0.16 mgm. down to 0.02 mgm., decreasing amounts of bronchoconstriction were produced (Figure 2). The decrease in vital capacity produced by identical amounts of histamine injected intramuscularly, regardless of the order of dosage, was similar, and appeared to rule out any notable cumulative effect of the drug (Table II). Four consecutive doses of 0.06 mgm. of histamine injected intramuscularly at 30-minute intervals produced similar amounts of reduction in the vital capacity (Figure 3). Slightly accelerated histamine effects occurred after several intramuscular injections in the same region, and it was felt that continued local vasodilation due to repeated histamine injections resulted in more rapid absorption. The flush and headache following the histamine injections appeared no more severe in asthmatic subjects than in normals.

TABLE I

The effect on the vital capacity of histamine given parenterally to asthmatic subjects

Patient	Age	Sex	Histamine intramuscularly			Histamine intravenously		
			Dosage	Reduction in vital capacity		Dosage	Reduction in vital capacity	
			mgm.	*ml.*	*per cent*	*mgm.*	*ml.*	*per cent*
J. D.	16	F	0.08	420	15	0.02	1,944	63
B. Y.	72	M	0.12	720	30	0.02	836	34
V. B.	49	M	0.16	408	9	0.02	2,874	68
C. C.	48	M	0.16	700	23	0.03	1,160	35
A. L.	33	M	0.20	732	14	0.03	1,338	27
M. K.	54	F	0.08	313	16	0.03	659	32
J. H.	38	M	0.08	628	11	0.02	1,498	28
B. R.	29	F	0.08	982	33	0.02	2,048	71
P. W.	26	M	0.02	no change		0.03	no change	
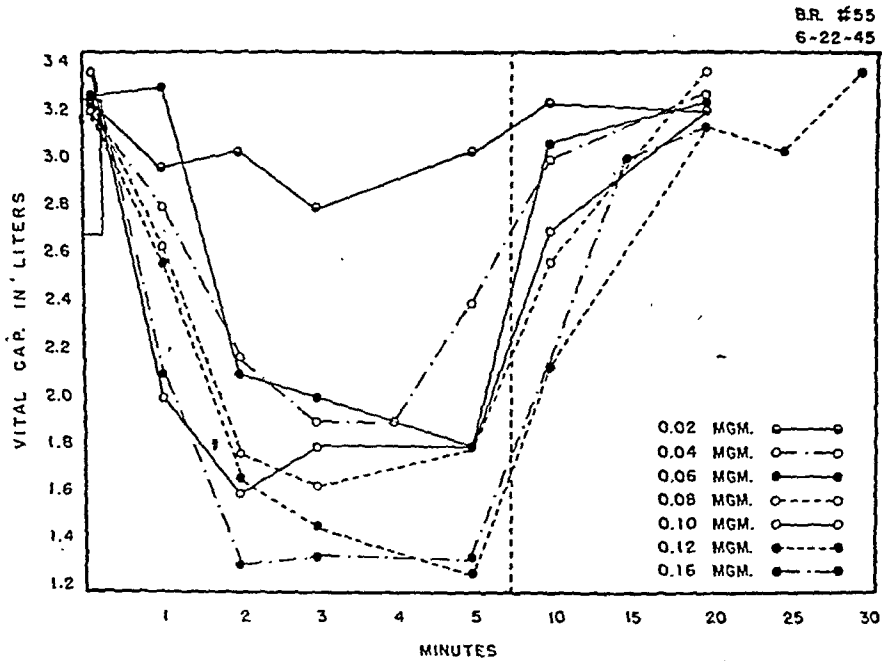
Fig. 1. Effect of I.M. Histamine. Gradually Increased Dosage
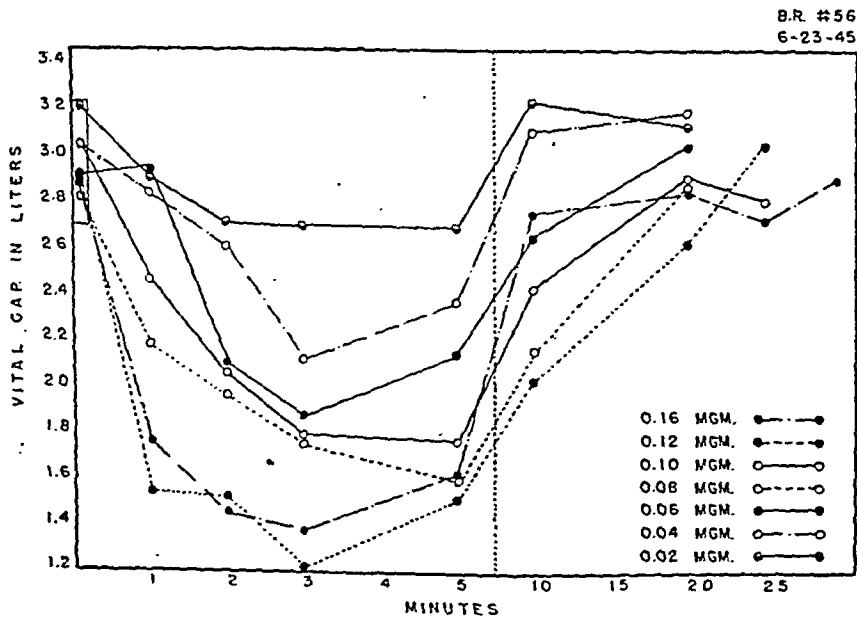
Fig. 2. Effect of I.M. Histamine. Gradually Decreased Dosage

TABLE II

Comparison of the effect on the vital capacity of progressively increasing and progressively decreasing the amounts of histamine injected intramuscularly in an asthmatic subject

Histamine intramuscularly	Vital capacity	
	Increasing dosage	Decreasing dosage
mgm.	ml.	ml.
control	3,240	3,200
0.02	(1) 2,800	(7) 2,700
0.04	(2) 1,922	(6) 2,135
0.06	(3) 1,812	(5) 1,890
0.08	(4) 1,650	(4) 1,620
0.10	(5) 1,600	(3) 1,780
0.12	(6) 1,297	(2) 1,225
0.16	(7) 1,318	(1) 1,380

Numbers in parentheses refer to the order of injection.

INTRAVENOUS HISTAMINE

After sensitivity of the tracheobronchial tree to histamine by the intramuscular injection had been determined in the reactive subjects, the drug was then given intravenously. Depending on the weight of the patient, the degree of tracheobronchial sensitivity, and the degree of side reactions, doses of from 0.01 to 0.04 mgm. of histamine were injected. Patients weighing less than 50 kgm. were ordinarily given not more than 0.02 mgm. of histamine intravenously in a single injection.

As might be expected when histamine was given by this route, bronchoconstriction was more marked, and appeared more quickly. A definite decrease in vital capacity could be demonstrated within 9 seconds after the intravenous injection of 0.03 mgm. of histamine. Since the arm-to-tongue circulation time varied from 12 to 20 seconds with histamine given intravenously, it was felt that this rapid reaction represented an effect from diffusion through the pulmonary artery and vein. The most marked effect was found to occur 30 seconds after the completion of the injection. It wore off very rapidly, so that in 5 to 10 minutes the vital capacity had returned nearly to the control levels. For example, in one test the injection of 0.02 mgm. of histamine intravenously resulted in a drop in vital capacity of 1,960 ml.. from the control level of 3,240 ml. to 1,280 ml. in 30 seconds. By 1½ minutes after the injection the vital capacity had increased to 1,780 ml., and at 2½ minutes had risen to 1,905 ml. A gradual return to the control level then occurred in 20 minutes.

With successive intravenous injections of similar amounts of histamine at 30-minute intervals, similar amounts of bronchoconstriction took place, but there appeared a tendency for the effect to wear off a little more quickly with each injection.
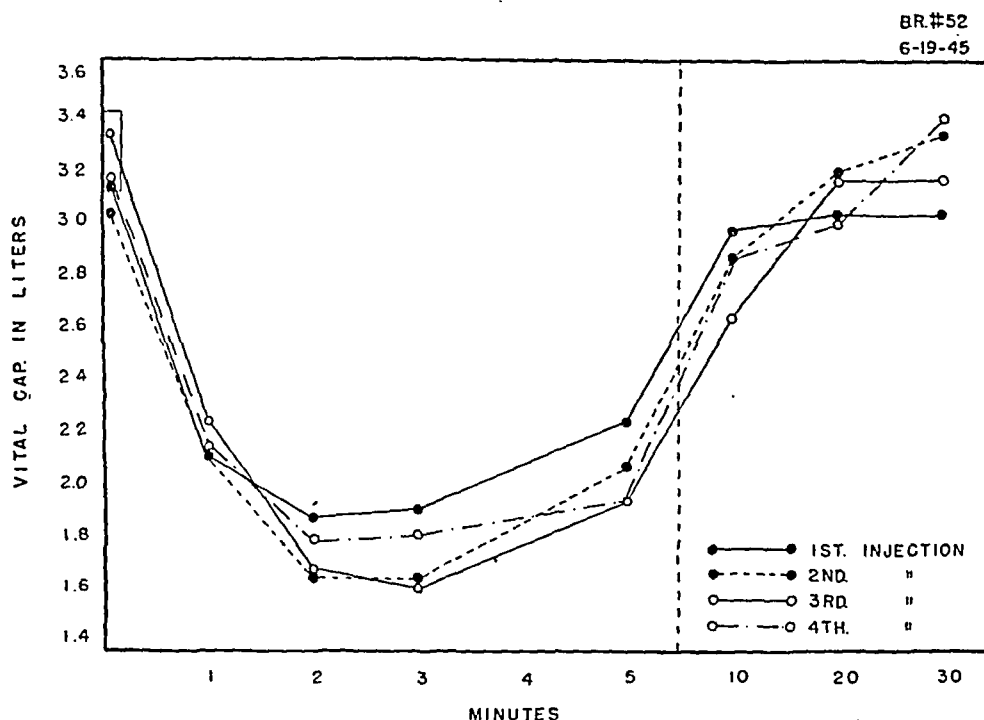
FIG. 3. EFFECT OF 0.06 MGM. I.M. HISTAMINE EVERY 30 MINUTES
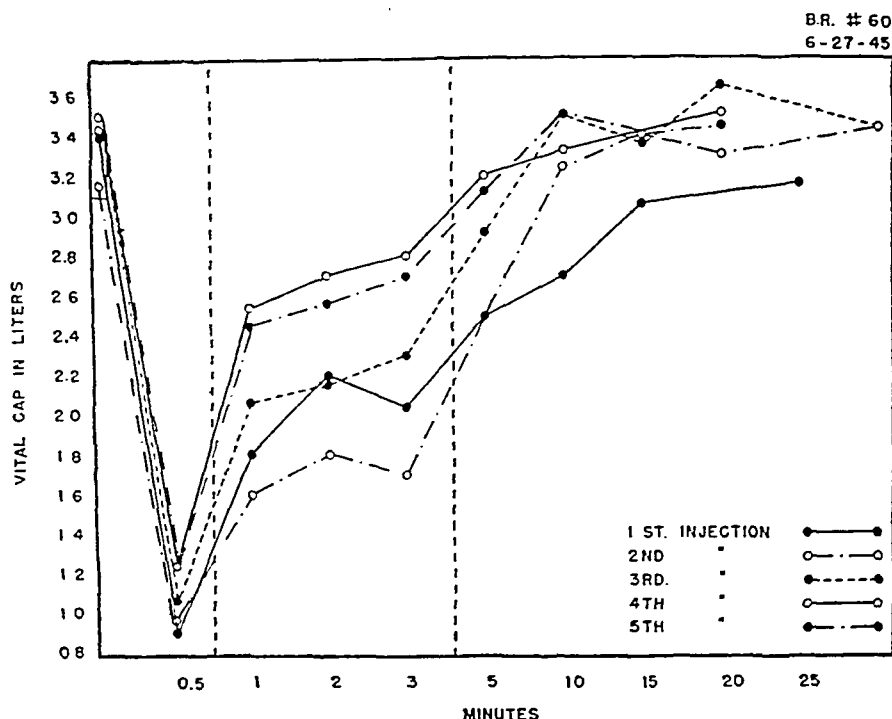
FIG. 4. EFFECT OF 0.04 MGM. I.V. HISTAMINE EVERY 30 MINUTES

For example, in subject B. R., 5 successive intravenous injections of 0.04 mgm. of histamine, at 30-minute intervals, resulted in decreases of 2,470 ml., 2,205 ml., 2,355 ml., 2,175 ml. and 2,225 ml., respectively, in the vital capacity (Figure 4). In order to determine whether additive amounts of histamine would have effect while the tracheobronchial tree was still reacting to a preceding injection of histamine the following tests were performed. In one instance, after the injection of 0.01 mgm. of histamine had reduced the vital capacity to 1,855 ml. in 30 seconds, the injection was repeated 30 seconds later, and a further reduction in the vital capacity to 1,615 ml. resulted. In another instance the second injection was given 1½ minutes later, and again a further decrease in vital capacity occurred. However, when the total dosage of 0.02 mgm. was given in a single injection, a greater reduction in vital capacity was achieved with a fall to 1,280 ml. (Figure 5). These studies indicate, then, that during any period of observation, similar amounts of bronchoconstriction can be produced when identical amounts of histamine are injected either intravenously or intramuscularly. It was a matter of great interest to determine whether this sensitivity was variable,

and it was soon noted that the degree of sensitivity varied with the severity of asthmatic symptoms. Occasionally an increase in sensitivity would manifest itself by a delay in the appearance of the greatest amount of bronchoconstriction. For example, after the intravenous injection of histamine, the greatest decrease in vital capacity would be noted at 1½ minutes, rather than at 30 seconds, and the return towards the normal range would also be delayed. Incidentally, it was also observed that the relief afforded by sympathomimetic amines against histamine bronchoconstriction was less at these times. In one instance, the intravenous injection of 0.02 mgm. of histamine reduced the vital capacity from 2,884 ml. to 836 ml., a drop of 2,048 ml., and 1½ hours were required before it returned to near the control range.

An irregular but definite bronchoconstriction occurred when 0.35 mgm. of histamine was placed under the tongue. The decreased vital capacity returned to normal after the mouth had been rinsed with saline. The nebulization of 1:1,000 histamine into the lung also produced a reduction in vital capacity. Four inhalations of 1:1,000 solution of histamine by nebulizer produced a notable reduction in vital capacity (Figure 6).
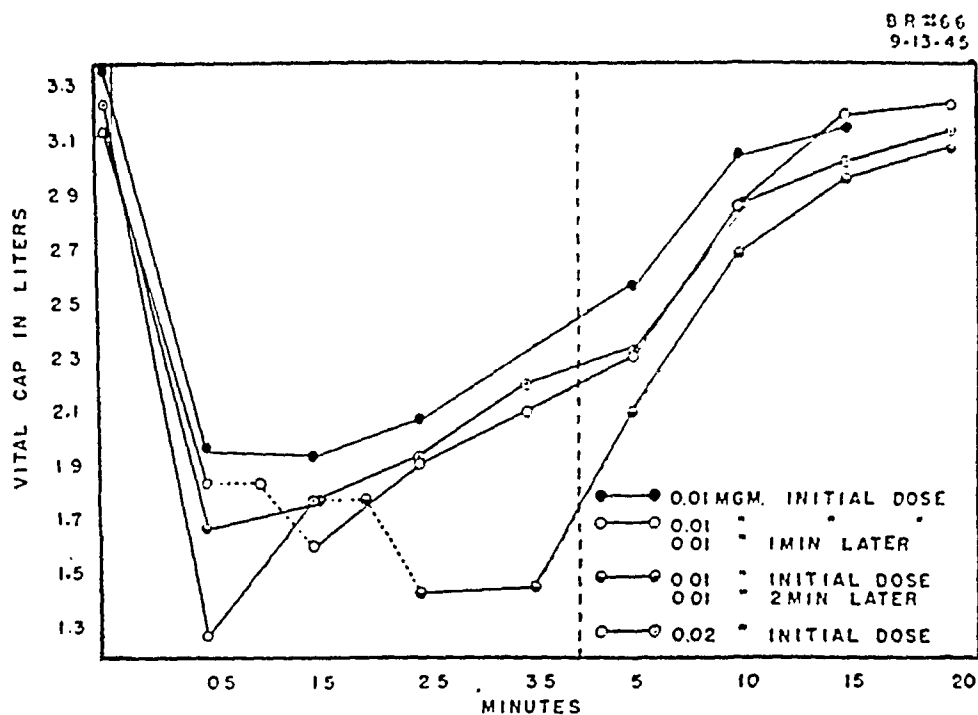
FIG. 5. EFFECT OF REPEATED DOSES I.V. HISTAMINE
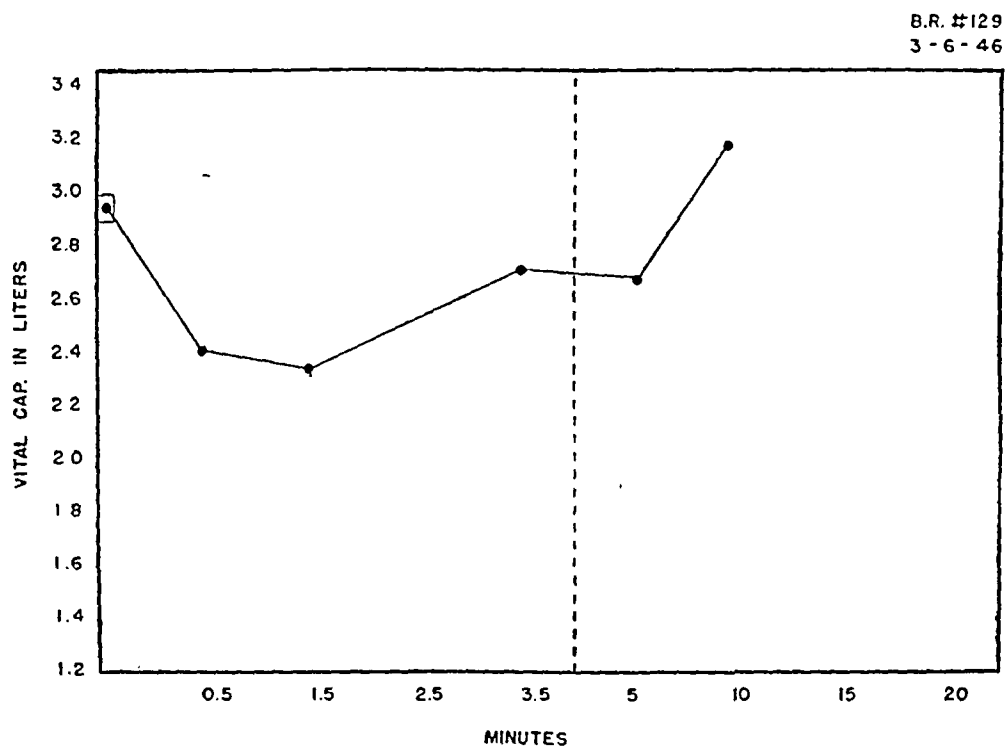
FIG. 6. EFFECT OF NEBULIZATION OF 1:1,000 HISTAMINE

The type of reaction resembled that produced by the intravenous administration of the drug.

SUMMARY

1. The reaction of the tracheobronchial tree to histamine was investigated in 10 normal subjects, 10 patients with a history of severe allergic tendencies, and 9 patients with varying degrees of bronchitis, emphysema and asthma. In the first 2 groups, no notable reduction in vital capacity was observed after the intramuscular injection of a dose of 0.36 mgm., or the intravenous administration of a dose of 0.02 to 0.03 mgm. of histamine. In the third group, the sensitivity of the tracheobronchial tree to histamine in asthmatic subjects was confirmed in 8 of the 9 patients. This sensitivity was found to vary from patient to patient, and with the degree of asthma.

2. During any one period of observation, quantitatively similar amounts of bronchoconstriction, as measured by a decrease in the vital capacity, could be produced by identical amounts of histamine injected at intervals by either the intramuscular or intravenous route. Bronchoconstriction may also be induced by administering histamine under the tongue, or by nebulization.

3. No evidence was adduced to show that the systemic reactions to histamine in asthmatic subjects differed from the systemic reactions in normals.

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THE EFFECT OF ANTIHISTAMINE SUBSTANCES AND OTHER DRUGS ON HISTAMINE BRONCHOCONSTRICTION IN ASTHMATIC SUBJECTS [1]

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The ability of antihistamine substances, sympathomimetic amines and other drugs to counteract histamine bronchoconstriction in animals has received widespread attention (1 to 10). Such tests have not been applied to normal human subjects because of failure to demonstrate significant amounts of bronchoconstriction after doses of histamine that may be safely administered. However, the occurrence of bronchoconstriction in many asthmatic patients following the administration of histamine has been confirmed (11). It was also discovered that this sensitivity to histamine varied from person to person and in the same individual with the severity of the asthmatic symptoms. Moreover, during any one period of study, repeated intramuscular or intravenous injections of identical quantities of histamine produced similar amounts of bronchoconstriction. Thus, the tracheobronchial reaction to histamine in a sensitive subject provides a means of assaying the effectiveness of various antihistamine preparations, as well as of other drugs, in counteracting this type of bronchoconstriction.

METHODS

The methods used in this study were similar to those described in a previous communication (11). Bronchoconstriction was demonstrated by a decrease in the vital capacity. The subjects were patients with mild continued asthma, all of whom had been studied previously and found to have sensitivity of the tracheobronchial tree to histamine. One patient, B. R., was again very cooperative, and we are grateful to her for the large number of studies made with her assistance. Control reactions to histamine were ascertained before any counteracting drug was administered, and in no instance was more than one such drug given during any period of study. All doses of histamine were injected intravenously, unless otherwise noted.

RESULTS

Benadryl [2] (B-dimethylaminoethyl benzhydryl ether hydrochloride) was synthesized by Rieveschl and Huber. Loew and his co-workers (1, 2) demonstrated that it has a potent antihistamine effect in animal experiments. Clinical reports (12) also indicate that it has been particularly effective against allergic symptoms thought to be histaminic in origin. In these reports, the adult dose of benadryl varies from 50 to 500 mgm. daily. In the present study, smaller amounts of the drug were used in order to determine the duration of its antihistamine effect on the respiratory tract against histamine bronchoconstriction.

In subject B. R., the vital capacity was reduced by a control injection of 0.02 mgm. of histamine from 3,020 ml. to 1,231 ml., a drop of 1,789 ml., and after the measurements had returned to the resting levels, a dose of 10 mgm. of benadryl in 16 ml. normal saline was injected intravenously during a period of 1½ minutes. No side effects from the injection were noted. Two and a-half minutes after the injection was completed, a second intravenous dose of 0.02 mgm. of histamine produced a drop in vital capacity of only 721 ml. With successive injections of 0.02 mgm. of histamine at half-hourly intervals, approximately the same amount of protection was afforded (Figure 1). The headache, flush and taste in the mouth, which previously occurred as side reactions after the injection of histamine, were also definitely diminished in intensity.

In another study on B. R. the control dose of 0.02 mgm. of histamine reduced the vital capacity from 2,999 ml. to 1,170 ml., a drop of 1,829 ml. When a dose of 30 mgm. of benadryl in 50 ml. of normal saline was injected intravenously during a period of 5 minutes, the patient noted a slight

[2] Parke Davis and Company.

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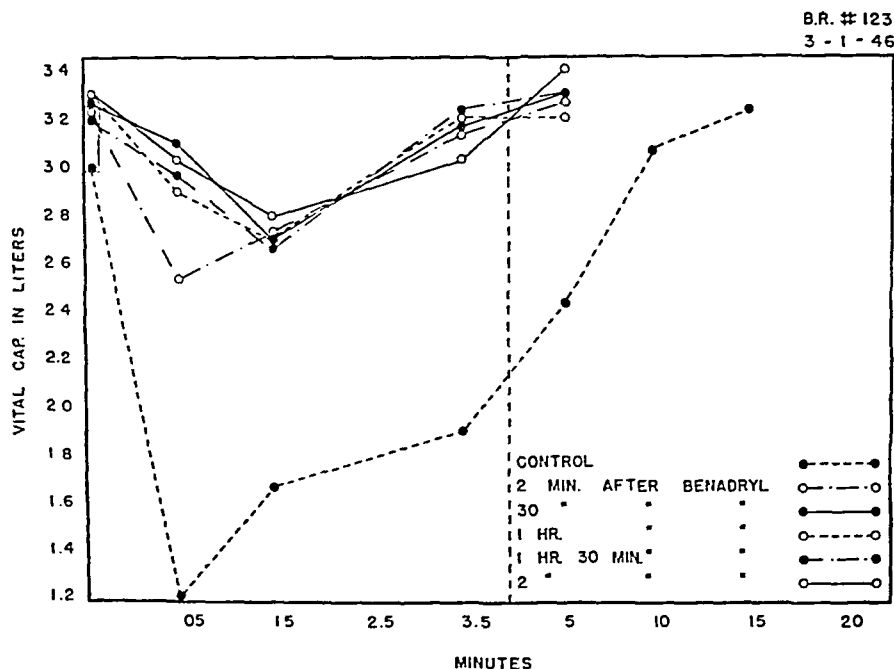
FIG. 1.   EFFECT OF 0.02 MGM. I.V. HISTAMINE AFTER 10 MGM. BENADRYL I.V.

sensation of giddiness, and a mild pressor effect was found in the arterial pressure, especially in the diastolic level.   Four minutes after the injection of benadryl was completed, the protection against the bronchoconstrictive and also the systemic effects of 0.02 mgm. of histamine was almost complete.   Successive injections of 0.02 mgm. of histamine at half-hourly intervals over a period of 3½ hours showed a diminishing protection during the last 2 tests.   Even then the drop in vital capacity at the 3½-hour test measured only 711 ml., compared to the control decrease of 1,829 ml. prior to the administration of benadryl (Figure 2).

In patient V. B. a dose of 30 mgm. of benadryl in 50 ml. of normal saline, given intravenously, afforded complete protection against the bronchoconstrictive and systemic effects of 0.02 mgm. of histamine administered by vein, 3 minutes after the injection of benadryl was completed.   In addition, the resting vital capacity was increased above previous control levels through the action of the benadryl.   This same patient also obtained clinical relief from his asthmatic symptoms by 50 mgm. capsules of benadryl taken by mouth.   This is of interest in view of the generally disappointing clinical results reported from treatment of asthma with benadryl (12).   It is possible that the tracheo-

bronchial reaction to histamine may provide a means of determining which patients will obtain relief clinically by the administration of benadryl. Further studies are in progress to establish whether there is a correlation between the effectiveness of benadryl in relieving the clinical symptoms of asthma and in counteracting histamine bronchoconstriction.

Pyribenzamine hydrochloride [3] (N'-pyridyl-N' benzyl-N dimethyl ethylenediamine monohydrochloride) is another substance that has marked ability to counteract the pharmacological effects of histamine.   Clinical studies have also shown it to be effective in relieving many allergic disturbances. Three of our asthmatic subjects were given 50 mgm. doses of pyribenzamine hydrochloride by mouth, and varying results were obtained.   Subject V. B. showed almost complete protection against the systemic, as well as the bronchoconstrictive, effects of 0.02 mgm. of histamine 1½ hours after the oral administration of 50 mgm. of pyribenzamine hydrochloride.   One-half hour after the drug was ingested, 0.02 mgm. of histamine produced a decrease of 3,189 ml. in the vital capacity, compared to the control decrease of 2,874 ml., but this decrease did not occur at the

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[3] Ciba Pharmaceutical Products, Inc.

30-second test, and instead occurred at the 1½-minute test, indicating a delay in the onset of the bronchoconstriction. One hour after pyribenzamine was given, a repeat injection of 0.02 mgm. of histamine again failed to produce any decrease in vital capacity at the 30-second interval, while at the 1½-minute test there was a reduction. This time, however, the reduction measured only 899 ml. A third injection of 0.02 mgm. of histamine 1½ hours after pyribenzamine hydrochloride failed to produce any significant reduction in the vital capacity (Figure 3). Thus, in this patient the drug apparently acted first to delay the onset of histamine bronchoconstriction, and finally to provide almost complete protection against both its bronchoconstrictive and systemic effects. Patient B. R., who had previously shown marked antihistamine protection from intravenous benadryl, failed to show any protection against the bronchoconstrictive or systemic effects of 0.01 mgm. of histamine given intravenously ½ hour and 1 hour after the ingestion of 50 mgm. of pyribenzamine hydrochloride. Subject A. L., on the other hand, 1½ hours after the oral ingestion of 50 mgm. of pyribenzamine, exhibited both a slight amount of systemic protection from the effects of 0.02 mgm. of histamine, in that the flush, headache

and taste in the mouth were less prominent, and a definite respiratory protection, in that histamine produced a drop of only 825 ml. in the vital capacity as compared with a decrease of 1,557 ml. in the control reaction to histamine prior to the administration of the pyribenzamine.

Atropine has been known to alter the gastric secretion evoked by histamine, and it was a matter of interest to determine what change it might produce in the reaction of the tracheobronchial tree to histamine in asthmatic individuals. In subject B. R., 4 inhalations from a nebulizer containing a 1 : 1,000 solution of histamine produced a decrease of 574 ml. in the vital capacity. Ten minutes following the intravenous administration of 1.2 mgm. of atropine sulfate, 4 inhalations were again taken, but failed to produce any bronchoconstriction. Twenty minutes after the atropine was given, 0.02 mgm. of histamine was injected intravenously. At the 30-second test the vital capacity had fallen 1,148 ml., but by 10 minutes had returned to the control range, a response less than might be expected from the amount of histamine injected (Figure 4). In this patient, therefore, atropine sulfate appeared to give some protection against the bronchoconstrictive effects of histamine.

Theophylline with ethylenediamine has been
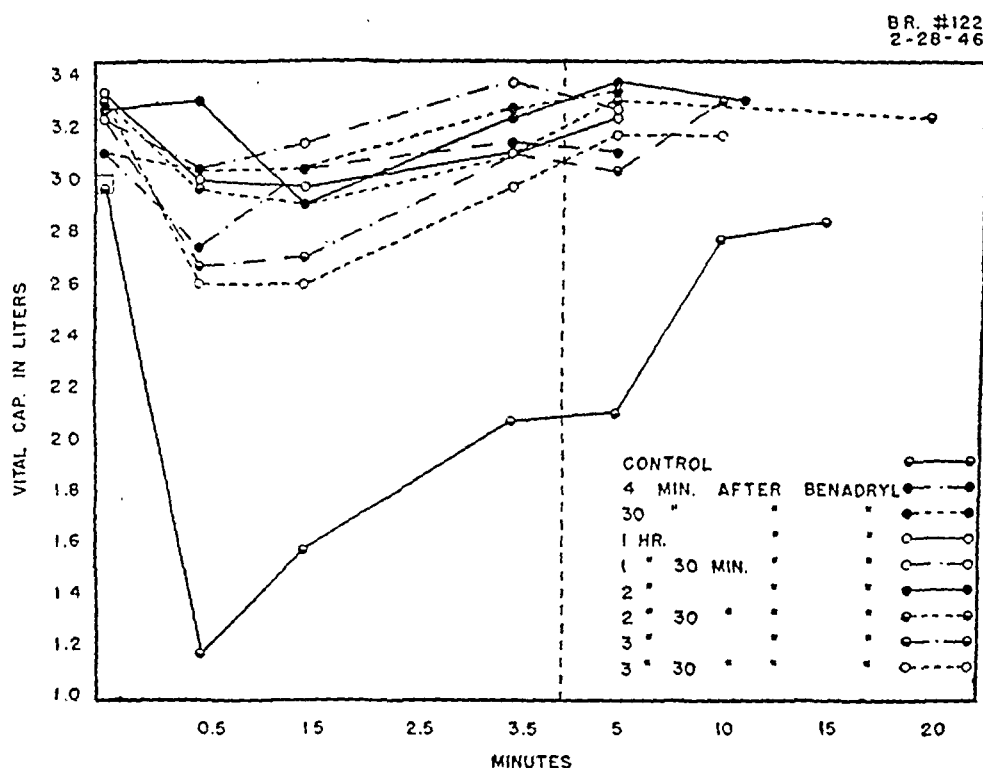
FIG. 2. EFFECT OF 0.02 MGM. I.V. HISTAMINE AFTER 30 MGM. BENADRYL I.V.
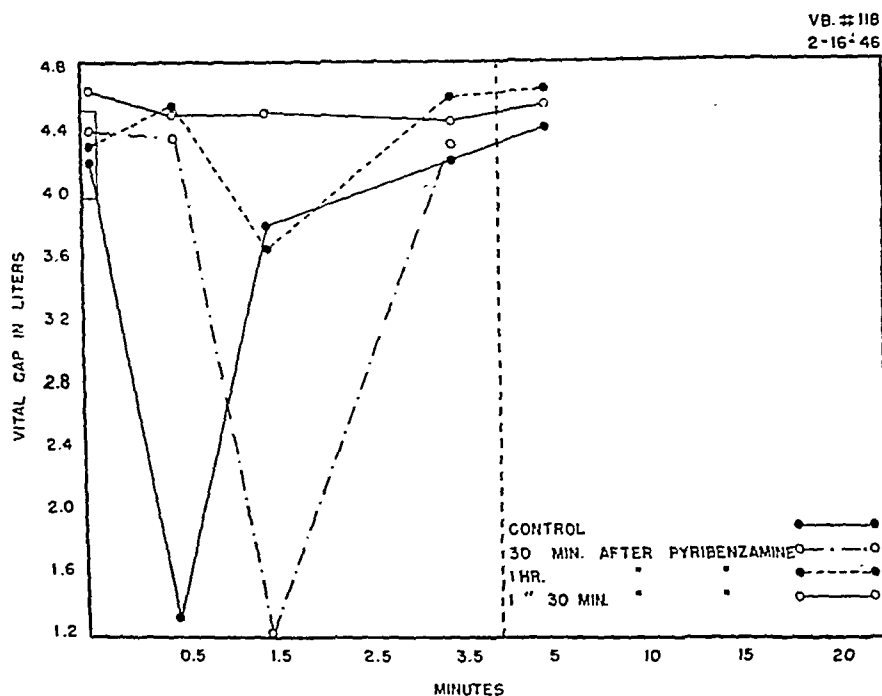
FIG. 3. EFFECT OF 0.02 MGM. I.V. HISTAMINE AFTER 50 MGM. PYRIBENZAMINE P.O.
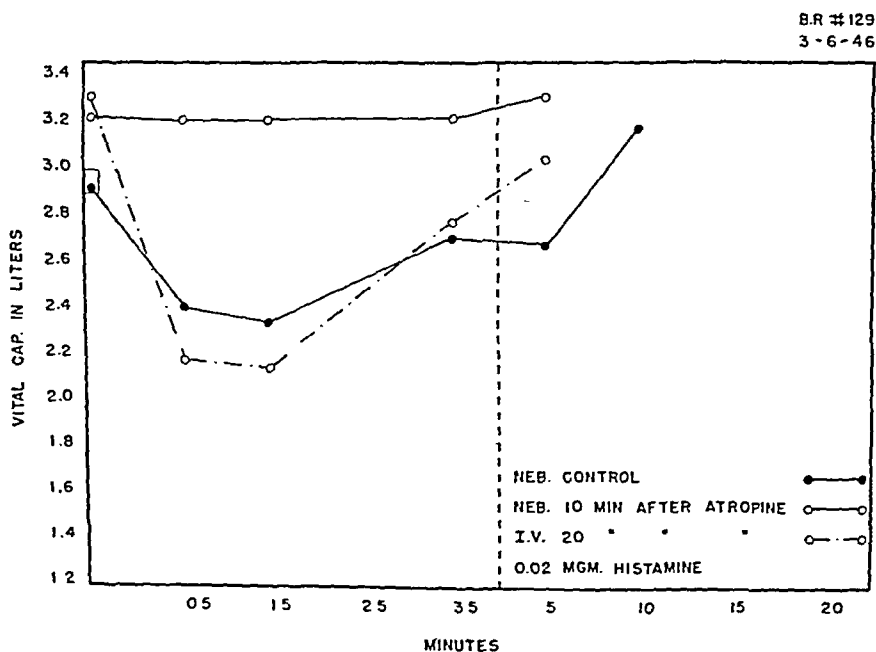
FIG. 4. EFFECT OF HISTAMINE AFTER 1.2 MGM. ATROPINE SULFATE I.V.

generally considered a potent bronchodilating drug when administered intravenously. It has also been demonstrated to provide protection against the tracheobronchial activity of histamine in animals. In subject B. R., during a control period, the in-

jection of 0.02 mgm. of histamine resulted in a decrease of 1,442 ml. in the vital capacity from an initial level of 2,926 ml. With a slow return over 30 minutes towards the previous levels of the vital capacities, this reaction indicated an increased sen-

sitivity of the tracheobronchial tree to histamine at this time. A dose of 500 mgm. of theophylline with ethylenediamine diluted in 20 ml. of normal saline was then slowly given intravenously. As a result of the injection, the resting vital capacity was increased by 396 ml. over the previous control tests. Fifteen minutes after the injection of theophylline with ethylenediamine was completed, a dose of 0.02 mgm. of histamine was again administered, and a drop in vital capacity of only 156 ml. occurred. However, because of the increased resting vital capacity this drop did not bring the vital capacity measurements below those in the previous control series (Figure 5). This appears to confirm experimental work in animals showing a marked protection from theophylline ethylenediamine against histamine bronchospasm.

As expected, epinephrine gave rapid protection against histamine bronchoconstriction. Control injection of 0.04 mgm. of histamine intravenously produced a drop in vital capacity of 2,225 ml. When the vital capacity had returned to the resting range, a dose of 0.3 ml. of 1:1,000 epinephrine was injected into the deltoid. Ten minutes later 0.04 mgm. of histamine produced a decrease of only 205 ml. in the vital capacity. However, as a result of an increased resting vital capacity due to the epinephrine, the drop after the injection was

well within the range of previous control vital capacities. This protection persisted for 1½ hours and then was lost, since at 2 hours and 2½ hours the same amount of histamine caused decreases in vital capacity only slightly less than those produced in the control state (Figure 6). In order to determine how small an amount of epinephrine might furnish protection in one subject, J. D., 0.1 ml. of 1:1,000 epinephrine was injected into the deltoid muscle. Ten minutes later, when a dose of 0.02 mgm. of histamine was injected, it produced a drop of only 485 ml. in the vital capacity as compared with the control drop of 1,935 ml. A half hour after the epinephrine was given, histamine produced a decrease of 815 ml. in the vital capacity, while at 1 hour it caused a decrease of 1,485 ml., indicating that the protective effect of the epinephrine was nearly dissipated. During a period when sensitivity to histamine was great in subject B. R., 0.4 ml. of 1:1,000 epinephrine gave less protection against histamine bronchoconstriction than it had on previous occasions, and the protection disappeared more rapidly (Figure 7). Epinephrine also protected to some extent against the flush after intravenous histamine, but did not affect the headache.

Ephedrine sulfate produced a marked protection against the tracheobronchial effect of histamine,
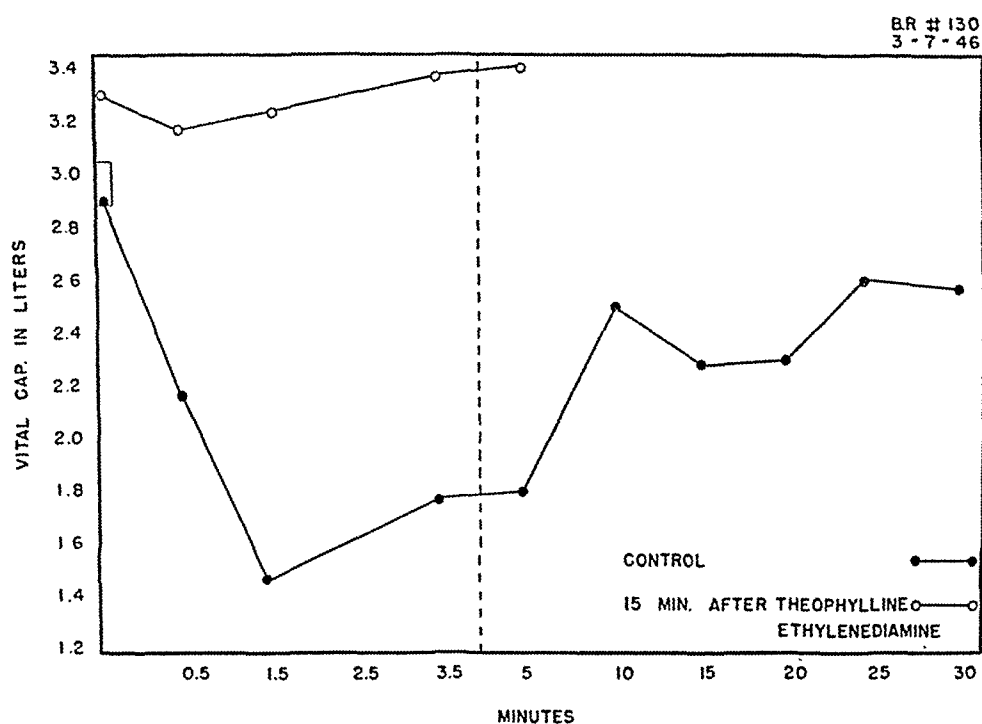
Fig. 5. Effect of 0.02 mgm. I.V. Histamine After 500 mgm. Theophylline Ethylenediamine I.V.
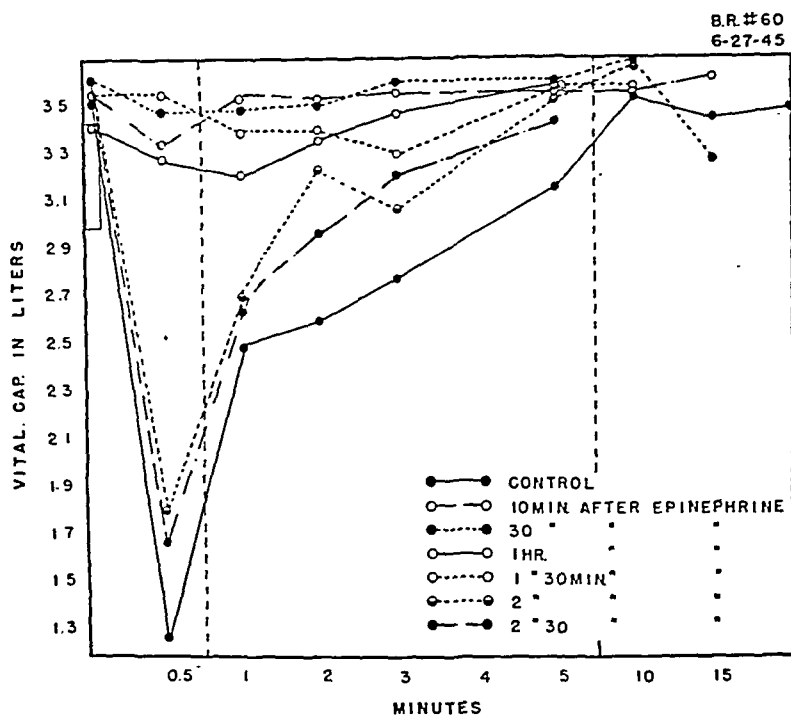
FIG. 6. EFFECT OF 0.04 MGM. I.V. HISTAMINE AFTER 0.3 ML. 1:1,000 EPINEPHRINE I.M.
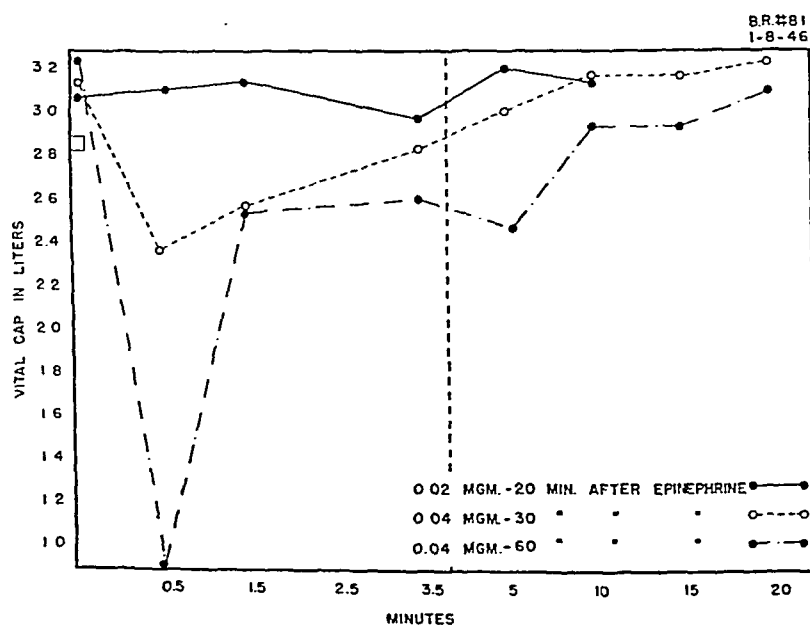
FIG. 7. EFFECT OF I.V. HISTAMINE AFTER 0.4 ML. 1:1,000 EPINEPHRINE I.M.

but the onset of protection was more gradual than that afforded by epinephrine. After 0.04 mgm. of histamine had been shown to produce a drop in the control vital capacity of 2,100 ml., a dose of 31 mgm. of ephedrine sulfate was given intra-muscularly in the deltoid. Ten minutes later 0.04 mgm. of histamine caused a drop of 1,355 ml. in the vital capacity. One-half hour after ephedrine sulfate, 0.04 mgm. of histamine caused a decrease of only 440 ml., and in 1 hour, a decrease of 315

ml. in the vital capacity. Injections of histamine at 2 and 3 hours after the ephedrine sulfate had practically no effect on the vital capacity determinations (Figure 8). These results appeared to bear out what we know about the clinical effects of ephedrine sulfate by showing that there was a gradually increasing action over a period of an hour, and then a sustained effect. As yet, we have not determined how long the effect may persist.

SUMMARY

1. Previous studies have demonstrated that quantitative amounts of bronchoconstriction may be produced in certain asthmatic subjects by parenteral histamine. In the present communication, attention was given to the ability of certain antihistamine substances and other drugs to protect against this bronchoconstriction. The degree of bronchoconstriction after given doses of histamine was measured by recording the decrease in the vital capacity.

2. Benadryl administered intravenously produced a remarkable, rapid protection against both the systemic and bronchoconstrictive effects of parenteral histamine. Pyribenzamine hydrochloride given orally in 50 mgm. doses produced a slow and more irregular protection. No comparison of the 2 preparations could be made, since they were given by different routes, but it appears that

with further study such a comparison can be made. In addition, further information should be obtained concerning the time of onset, extent and duration of effectiveness of the oral preparations.

3. Atropine sulfate furnished complete protection against the bronchoconstrictive effects of nebulized histamine in one instance, but only partial protection against intravenously administered histamine.

4. Theophylline with ethylenediamine given by the intravenous route afforded prompt and potent protection against the tracheobronchial effects of intravenous histamine.

5. Intramuscular epinephrine gave prompt and complete protection against the bronchoconstrictive effects of histamine, while intramuscular ephedrine sulfate also produced complete protection. With ephedrine, however, the protection was slow in onset, and only became complete 2 hours after the drug was administered. It appears that this method of study provides a means of measuring the bronchodilator activity of the various sympathomimetic amines.

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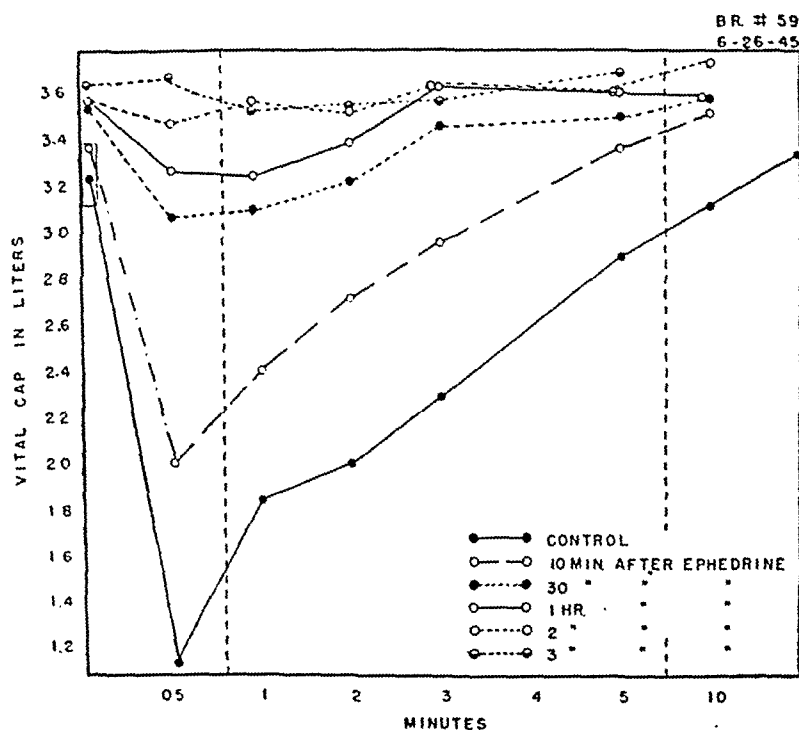
FIG. 8. EFFECT OF 0.04 MGM. I.V. HISTAMINE AFTER 31 MGM. EPHEDRINE SULFATE I.M.

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COMPLEMENT IN INFECTIOUS DISEASE IN MAN [1]

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Since Ehrlich and Morgenroth (1) made the observation that antibody and complement are independent entities, and Wassermann (2) advanced the hypothesis that serum complement concentration serves as a measure of general resistance, many attempts have been made to elucidate the role of complement in acute and chronic diseases. Longcope (3) held that "terminal infection in chronic disease is probably the direct result of the diminished state of bacteriolytic complement."

Dick (4) noted that in cases of *pneumonia*, complement was low before crisis, and high on the second to third day following crisis. The recent work of Rutstein and Walker (5) also points to a diminished complement in patients with *pneumonia* at the time of their admission to the hospital. These authors further remarked that the sera of 7 of 12 patients with pneumonia, tested immediately after the administration of antipneumococcal serum, showed diminished complement titers. The sera of 6 of 11 patients with *serum disease* were also found by these authors to have decreased complement titers. Goussev (6) and Wendstrand (7) reported, however, an increase in complement in the sera of patients with pneumonia.

Gunn (8) found complement always present during the course of enteric fever, although in greater amount throughout the period of pyrexia than during convalescence. He remarked that a diminution of titer in favorable cases coincided with the production of antibody. According to Gunn, complement and antibody are not produced in any fixed ratio to one another.

In *erysipelas* Gunn (8) found complement present in greater amount during the acute stage of illness than during convalescence. Keefer and Spink (9) also noted greater fluctuations of complement titer in this disease than observed in controls without infections.

In cases of *tuberculosis* a low complement titer has been observed by Gaudeau (10), an elevation by Goussev (6), while Hanson (11) reported little or no change. Meersseman and Perrot (12), in their turn, claimed that a low complement titer in tuberculosis is an unfavorable sign.

Bertin (13) and Wendlberger and Volavsek (14) reported reduction of complement titer in some cases of *syphilis*.

In cases of *yellow fever*, da Costa Cruz (15) claimed that a low complement titer is of diagnostic significance. He based this conclusion on a study of 103 cases, 93.6 per cent of which he defined as being of low titer. During convalescence, previously diminished titers were found to increase rapidly toward normal.

Thompson (16) found a diminution of complement in the early stages of *Variola*, with a rapid return to normal in cases unattended by secondary infection. A failure of the titer to return to normal was observed in cases with secondary infection, and a progressive decrease of complement was also observed in cases complicated by terminal septicemia.

A reduction of complement titer during *malarial paroxysms* was reported by Cathoire (17), Vincent (18), and Wendlberger and Volavsek (14).

Hadjopoulos and Burbank (19) stated that the complement content of the serum of a patient with infectious disease shows considerable variation from the normal, and that "such variations have been demonstrated by us to have prognostic significance." They found that with the onset of infection a steady rise in the production of complement occurs; however, this stage passes rapidly into a so-called "negative alexic phase" which they consider to be the "serologic shock period." An abnormal fall of complement then ensues. Further, according to these workers, the decrease of complement is probably due to an increased destruction of complement, or to its adsorption by proteolytic by-products of known anticomple-

mentary properties. During recovery and convalescence, "the ratio between complement production and destruction is reversed."

Others, notably Brinkmann (20) and Schuchardt (21), have failed to find sufficient changes of titer in pathological states to consider complement a measure of diagnostic or prognostic value.

With regard to laboratory animals, there is one instance in the literature which dramatically correlates complement titer with resistance to infection. In this instance, guinea pigs bred by Moore (22) were found to be deficient in complement and simultaneously highly susceptible to natural and experimental infections. This strain of guinea pigs, because of its innate lack of resistance to infection, is now extinct.

As pointed out above in the discussion of the work of Rutstein and Walker, complement titer may decrease as a result of the administration of antiserum. Thomas and Dingle (23) found this to be true also in the case of rabbits injected with a concentrated antimeningococcus serum, the result of which was the disappearance of hemolytic complement for as long as 24 hours. Meningococcal bacteremia, they found, persisted for a longer time in rabbits so treated than in animals receiving no antiserum. According to these authors, the disappearance of complement activity is due to the prozone effect, as well as to the anticomplementary action of the antiserum.

From a different viewpoint, the role of complement in infectious disease has been emphasized by the recommendations of numerous authors that serum containing complement be administered together with antiserum, as suggested, for instance, by Fairley and Stewart (24) and Kolmer (25) in cases of meningococcal infection.

This summary of complement in infectious diseases may be concluded with the statement of Osborn (26) that, "Very much more work is required before the clinical significance of complement estimations can be assessed, and it will require much investigation, which some might regard as of an academic nature, before this section of immunological chemistry can obtain any wide application in the diagnosis, prognosis, or treatment of disease."

It might be added that many of the apparent contraindications and inconsistencies encountered in the literature surveyed can be attributed to the lack of a uniform technique of complement titration, and to an inadequate definition of the limits of "normal" and "abnormal" complement titers.

The present paper is concerned with a detailed study of complement titers in 278 cases of various infectious diseases. This study is unique, in contrast to earlier work, in that it includes not only the determination of over-all complement titers, but the approximation of the titers of the individual complement components as well. Further, a beginning is made in the correlation of titer with serum protein concentrations, leukocyte count, and body temperature.

METHODS

The subjects of this study were patients in the Cleveland City Hospital, Contagious Division, or in the University Hospitals. In the majority of cases serial studies were made, with at least 2 blood samples being obtained from a patient during the course of hospitalization. In most cases the first blood sample was obtained on the day of admission prior to the use of therapeutic measures. This precaution was observed because of the fact that administered antiserum may depress the complement titer, although the authors, in control experiments, found that the highest blood levels of sulfonamide drugs and penicillin encountered in this study were without significant effect on the complement titer.

(a) *Collection of serum.* Blood was obtained by venous puncture, allowed to clot for several hours at 3 to 4° C., centrifuged, and the serum decanted and frozen at − 35° C. until used. The greatest number of specimens were titrated within a day of collection, others were titrated after storage for several days. Repeated trials showed that storage for this time at − 35° C. did not appreciably affect the complement titer.

(b) *Complement titration.* The overall complement titer, expressed in units per ml. of serum, is given in the summary tables, the unit being the minimum amount of serum which hemolyzes completely a standard dose of sensitized sheep red cells. The red cell substrate is made up as follows: about 5 ml. of defibrinated sheep blood is washed 4 times with 40 ml. portions of 0.9 per cent saline. Packed cells are then added to 100 ml. of saline so that a 1:20 dilution of the suspension gives a reading of 280 in a Klett-Summerson colorimeter. One hundred ml. of saline is now prepared so as to contain 20 units per ml. of hemolysin. This sensitizing solution is mixed rapidly with 100 ml. of the cell suspension; a 1:20 dilution of this mixture in saline should now give a reading of 140 in the colorimeter. Adjustments to obtain this reading are made either by addition of packed cells or by dilution with saline.

One volume of the test serum is now diluted with 4 volumes of saline (1:5). To 18 serological tubes are added amounts of the 1:5 serum dilution ranging from

0.01 ml. to 0.45 ml. with a 0.2 ml. Kahn pipette. Saline is added to bring the volume to 0.5 ml., and 0.5 ml. of the cell substrate is added with rotation of the tubes. The tubes are then incubated in a water bath for 30 minutes at 37° C. After incubation they are centrifuged at 4° C. for 5 minutes and the supernates compared visually with a previously prepared set of color standards.

Reactivation. The smallest amount of test serum found to yield barely a trace of hemolysis is employed for re-activation. Usually, normal sera require 0.02 ml. of a 1:30 dilution, but in many instances where the overall titer is low, as occurs in disease, higher quantities are required. Thus, in the case of a normal serum 0.02 ml. of the 1:30 dilution is pipetted into each of 5 tubes. The tubes then receive the previously prepared complement reagents in amounts adequate for reactivation, but avoiding anticomplementary effects. To the first tube is adde the "pH 5.4 − μ 0.02" supernate lacking C'1; the second receives the "pH 5.4 − μ 0.02" precipitate lacking C'2; the third receives Zymosan-treated serum which lacks C'3; and the fourth, NH₄OH treated serum lacking C'4. Generally, 0.3 ml. of each previously tested component is employed. The complement reagents were prepared from pooled sera which had an overall titer of 0.15 of 1:5 dilution. Therefore, in order to obtain at least 2 units of the reagents, they were diluted so as to correspond to a 1:5 dilution of the original serum. The fifth tube reacts as a control, and receives no complement reagent. Each tube is made up to 0.5 ml. volume with saline, and incubated for 30 minutes at 37° C. in a water bath.

In normal human serum the first component to disappear upon dilution is C'3, followed closely by C'2. At the point where these components disappear the serum still contains sufficient concentrations of C'1 and C'4 to produce 80 to 90 per cent hemolysis of the standard unit of red cell substrate. Accordingly, C'1 and C'4 may be said to be present in 8 to 9 times the effective concentration of C'2 and C'3.

(c) Determination of serum protein. If sufficient serum was available, protein determinations were performed by the refractometric method using a dip refractometer, the scale readings of which had previously been correlated with human serum protein concentration as determined by micro-Pregl nitrogen analysis. From time to time values were obtained for the same sera by nitrogen analysis, and always were found to be in good agreement.

(d) Clinical data. These were obtained from the hospital case records at the close of the laboratory work. During the course of the investigation none of the laboratory workers was familiar with clinical diagnosis, treatment, condition, etc., of the patient.

In all cases in which they were available for the day on which a blood sample for complement titration was taken, the total white blood cell count and the maximum body temperature of the patient were abstracted from the records.

RESULTS

a. The distribution of overall complement titers. Table I compares the distribution among 3 categories of complement titers obtained in a study of 248 cases of infectious disease and in 40 normals. From the distribution among this series of normals, as well as among previous series (28), complement titers of 25 to 50 units may be considered as "normal," titers below 25 units as low, and those above 50 units as high. Considered in these categories of titer, the distribution of titers in the diseased series is different from that in the normal series. Although the deviation from the normal of those sera showing a very low titer is more striking than the amount of deviation showed by those with high titers, there is enough departure from the normal in those with high titers to make the observation significant. The tendency toward low titers in the diseased series is even more significant, and the table shows that 30.6 per cent of the patients exhibited diminished complement titers. It may be further pointed out that about ⅓ of these, or 9.6 per cent of the total cases, showed titers less than 13 units per ml. of serum.

Among the separate diseases shown in the

TABLE I

Distribution of complement titers in 248 cases of infectious disease and in 40 normal sera

Disease	No. of cases	Lowest titer observed in course of disease		
		Percentage of cases showing the following titers		
		over 50 units*	25 to 50 units*	less than 25 units*
Scarlet fever	73	17.8	53.4	28.8
Epidemic meningitis	38	7.9	42.1	50.0
Measles	37	10.8	54.1	35.1
Pneumococcal infections of all types	36	19.4	61.1	19.5
Encephalitis (including all etiological types)	11	9.1	72.7	18.2
Typhoid fever	10	0.0	83.3	16.7
Erysipelas	7	42.9	42.9	14.2
Chicken pox	6	0.0	83.3	16.7
Subacute bacterial endo-carditis	5	0.0	60.0	40.0
Miscellaneous†	25	12.0	60.0	28.0
All cases	248	13.7	55.7	30.6
Normals	40	5.0	95.0	0.0

* Units per ml. of whole serum.
† Includes cases of rheumatic fever, gonococcal arthritis, naso-pharyngitis, peritonsillar abscess, influenza, and other upper respiratory infections.

table, cases of epidemic (meningococcal) meningitis present the most marked tendency toward a low complement titer, and cases of erysipelas the greatest tendency toward high titers. The distribution of titers in pneumococcal infections tends to be symmetrical about the normal range.

In analyzing the relationship of component titers to these changes in overall titer, it was found that in practically all cases diminution of complement titer was due primarily to decrease of C'4, and secondarily to decrease of C'2 and C'1. C'3 was apparently decreased only very slightly or not at all. On the other hand, all 4 components apparently participated in the shift toward increased titers.

b. Cases exhibiting very low complement titers. As seen in Table II, 12, or 4.3 per cent, of a total of 278 cases of infectious disease exhibited titers equal to or less than 10 units per ml. of serum. The most striking aspect of these complements, as seen in the table, was their almost complete loss of C'4 titer. C'2 titers were considerably reduced or absent in at least 7 of these instances, while C'1 titers were diminished, though not so sharply, in at least 4 cases. Very little change was encountered in the C'3 titer of any of these cases.

Of possible significance is the fact that 4 of these patients died, and that their lowest levels of complement titer occurred on the day or days preceding death. On the other hand, the low titers exhibited by patients who subsequently recovered were coincident with serious phases in the course

of the disease, and recovery was accompanied by a rapid return to a normal complement titer and to normal component titers. Such a course is exemplified in Case No. 1 shown in Table II. This patient, suffering with epidemic meningitis, was admitted to the hospital in a comatose state, and was found to have a titer of 10 units of complement per ml. of serum. Three days later, after the institution of treatment, the patient continued in a serious condition although she began to regain consciousness; nevertheless the complement titer had completely disappeared. Three days later the patient was fully on the road to recovery, and the complement titer had returned to a normal value of 36 units per ml. of serum.

c. Complement titers in fatal cases. Fourteen, or 5.0 per cent, of the total of 278 cases investigated died during the course of the study; of these, 8, or 2.9 per cent of the total number of cases, died without complications such as cancer, coronary disease, or other serious non-infectious diseases. Of the 8 having "uncomplicated" deaths, 4, or 50 per cent, had extremely low complement titers. Of the 6 dying with complications, none had low titers.

Table III shows the disposition of complement titers in 9 fatal cases of infectious disease. Besides the tendency toward low titers already noted, the high titers found in the 2 cases of Type III pneumococcal meningitis are noteworthy. The participation of the individual components in all of

TABLE II

Cases having 10 or less units of complement

Case no.	Diagnosis	Lowest C' titer in course of study	C'1	C'2	C'3	C'4	Remarks
		units per ml. serum					
1	Epid. men.	0		L		0	Recov. titer
3	Staph. men.	0		0		0	Died
38	Chronic fever of unknown etiol.	2				0	Recov. titer
86a	Epid. men.	0	N	N	N	0	Recov. titer
93	Pertussis, pn.	10	L	N	N	Tr	Died
117f	Strep., pn.	0	N	L	N	0	Recov. titer
191	Epid. men.	10	SL	0	N	0	Recov. titer
216	Post-measles enceph.	0	L	L	N	Tr	Died
240*	Epid. men.	0	N	N	N	Tr-0	Recov. titer
268c	Post-measles enceph., pn.	10	N	L	N	SL	Died
271a*	Epid. men., serum sickness	0	SL	Tr	SL	0	Recov. titer
276a*	Epid. men. later serum sickness	9	N	L	N	L	Recov. titer

* Cells agglutinated.
N = normal
L = low

SL = slightly low
0 = zero
Tr = trace

TABLE III

Complement titers in fatal cases

Case no.	Age	Diagnosis	Last C' titer	Date of last C' titration	Date of death	Remarks
	years		*units per ml. serum*			
3	29	Staph. men.	0	1-20	1-20	No C'2 and C'4
39	30	Type XII Pn. men.	31	2-8	2-10	
61	51	Epid. men.; uremia	23	2-16	2-23	Low C'4
64	30	Pn. Type III men.	83	2-16	2-18	All components higher
74	40	Pn. Type III men.	83	2-15	2-15	All components higher, particularly C'3
93	16 mon.	Pertussis pneum.; nephrotic synd.	10	2-20	2-23	C'1 low; C'4 trace
140	35	Lobar pneumonia	50	2-26	2-29	
216	31	Post-measles enceph.	0	3-7	3-8	C'1, C'2 low; no C'4
268	6	Post-measles enceph.	10	4-27	5-21	C'1 low; C'4 trace

these deviations of titer is the same as discussed in previous sections of this paper.

d. Serum protein concentration and complement titer. Because complementary activity is associated with serum proteins, an attempt was made to determine whether a relationship exists between complement titer and serum protein concentration. Table IV summarizes the relationship of 190 complement titers to 3 arbitrarily conceived categories of serum protein concentration. While it is difficult to define a "normal" range of serum protein concentration, the range of 6.5 to 7.4 per cent as given in the table has been found to be fairly normal with the refractometric method. In any event, the intent of the analysis is not so much to relate complement titers with so-called normal or abnormal serum protein concentrations, as to show the general distribution of titers over the encountered range of protein concentrations. The table reveals that serum protein concentration, in a general way, is a contributing factor in the determination of overall complement titer. A serum with low protein concentration tends in the direc-

tion of low titer, while a serum of high protein content tends toward high titer as opposed to low. Again, it is emphasized that these tendencies are modified by more significant factors which determine titer, inasmuch as complement components constitute only a very small fraction of the total serum proteins.

e. White blood cell count and complement titer. Table V shows the distribution of 181 complement titers among 4 arbitrarily defined categories of total leukocyte counts. It is apparent that no general relationship exists between cell count and titer, and that even a limited tendency is expressed in only one instance. This tendency is represented by the fact that 17.0 per cent of the sera showing less than 25 units of complement per ml. were obtained from patients having cell counts greater than 24,000 per cu. mm. This figure is in contrast to the extremely low percentages occurring otherwise in this range of leukocyte count. However, the occurrence of high white cell counts is not general for all infectious diseases, and this must be considered in evaluating the aforementioned

TABLE IV

Serum protein concentration and complement titer

Per cent protein	Sera having complement titers of:*						Total of all titers	
	Over 50 units		25 to 30 units		Less than 25 units			
	no.	*per cent*	*no.*	*per cent*	*no.*	*per cent*	*no.*	*per cent*
Less than 6.5	6	20.0	38	34.9	22	45.8	66	34.7
6.5 to 7.4	19	63.3	59	52.7	24	50.0	102	53.7
More than 7.4	5	16.7	15	13.4	2	5.2	22	11.6
Total of	30	100.0	112	100.0	48	100.0	190	100.0

* Units per ml. serum.

TABLE V

White blood cell count and complement titer

White cells per cu. mm.	Sera having complement titers of:[*]						Total of all titers	
	Over 50 units		25 to 50 units		Less than 25 units			
	no.	per cent	no.	per cent	no.	per cent	no.	per cent
12,000 and less	14	42.4	53	49.5	17	41.5	84	46.4
12,000 to 24,000	18	54.5	52	48.6	17	41.5	87	48.1
24,000 to 36,000	1	3.1	0	0.0	3	7.3	4	2.2
Over 36,000	0	0.0	2	1.9	4	9.7	6	3.3
Total of all cell counts	33	100.0	107	100.0	41	100.0	181	100.0

[*] Units per ml. serum.

tendency. As a matter of fact, almost all of the bloods having low complement titers and simultaneously high cell counts were *obtained from patients with epidemic meningitis*. On the other hand, in 2 cases of typhoid fever both the complement titer and white cell count were low. In other instances, including cases of scarlet fever, measles, and chicken pox, there was no relationship between titer and cell count.

f. Body temperature and complement titer. Since fever is a common phenomenon in infection, an attempt was made to correlate temperature and complement titer. Table VI relates 244 titers with the maximum body temperature of the patient on the day the blood sample was taken for complement titration. It is seen that there is the same distribution of temperatures for each category of complement titer, so that it may be said that there is no apparent relationship between body temperature and titer.

g. Examples of particular cases. Tables VII to IX summarize the data obtained in 3 cases of infectious disease, and serve to illustrate some of the changes in titer encountered. For the most part these tables are self-explanatory, although Case 117, the most fully studied case, deserves some comment. On admission, this patient was in serious condition and simultaneously his complement titer was also diminishing; in the course of the next few days he became comatose and at the same time his titer continued to decline, particularly the C'4 titer. In addition to penicillin, the patient received several blood transfusions and the complement titer increased somewhat; however the condition of the patient did not improve markedly. On 3-16-44, with the patient continuing in a dangerous state, his titer became zero with a simultaneous disappearance of C'4 titer. From that time on the patient began to improve until the time he was discharged. Three days before discharge his complement titer was fully restored concomitant with a restoration of C'4 titer.

TABLE VI

Maximum body temperature and complement titer

Max. temp. on day of blood sample	Sera having complement titers of:[*]						Total of all titers	
	Over 50 units		25 to 50 units		Less than 25 units			
°C.	no.	per cent	no.	per cent	no.	per cent	no.	per cent
36 to 38	13	38.2	45	32.2	25	35.7	83	34.0
38 to 39	9	26.5	41	29.3	18	25.7	68	28.0
39 to 40	5	14.7	28	20.0	13	18.6	46	18.8
40 to 41	7	20.6	25	17.8	14	20.0	46	18.8
Over 41	0	0.0	1	0.7	0	0.0	1	0.4
Total of all temperatures	34	100.0	140	100.0	70	100.0	244	100.0

[*] Units per ml. serum.

TABLE VII

J. R. Bronchopneumonia. Streptococcal Septicemia

Date*	Titer: complete hemolysis	C'1	C'2	C'3	C'4	Serum protein	Remarks
	units per ml. serum					*grams per cent*	
2-24-44	23					6.3	
2-29-44	13	SL	SL	N	N	6.4	25,000 units penicillin daily until 3-9-44
3-2-44	6	L	SL	N	L	7.8	500 ml. whole blood given
3-3-44	13	SL	SL	N	L	7.5	
3-6-44	12	SL	SL	N	L	6.8	500 ml. whole blood given on 3-7-44
3-8-44	12	SL	SL	SL	L	6.8	Sulfadiazine given from 3-11-44 to 3-16-44
3-16-44	0	SL	L	SL	0	5.7	Sulfadiazine level in blood: 3.6 mgm. per cent
4-1-44	31	N	N	N	N	7.0	

* Admitted 2-24-44—discharged, 4-4-44.
Key: N = normal titer
SL = slightly diminished
L = low, greatly diminished
0 = no titer

TABLE VIII

J. H.   Scarlet fever.   Measles

Date*	Titer: complete hemolysis	C'1	C'2	C'3	C'4	Serum protein	Remarks
	units per ml. serum					*grams per cent*	
3-8-44	56	SH	SH	SH	N	5.8	From 3-8-44 to 3-13, 18.5 grams sulfadiazine
3-16-44	56	SH	SH	SH	SH	7.1	From 3-19-44 to 3-23, 17 grams sulfadiazine
3-22-44	56	N	SH	SH	N	6.7	

* Admitted, 3-8-44; discharged, 3-25-44.
Key: N = normal titer
SH = slightly high titer, somewhat increased

TABLE IX

D.J. Epidemic meningitis, treated with antitoxin and sulfa drugs

Date*	Titer: complete hemolysis	C'1	C'2	C'3	C'4
	units per ml. serum				
2-22-44	2	L	L	.SH	Tr to 0
2-24-44	0	SL	L	SH	0
2-26-44	5	N	L	H	Tr to 0
3-7-44	23	N	N	SH	N

* Admitted, 2-22-44; discharged, 3-7-44.
Key: N = normal titer
SH = slightly high
H = markedly increased or high
SL = slightly low or diminished
L = very low, or greatly diminished
Tr = trace
0 = no titer

SUMMARY AND CONCLUSIONS

Without going into detail about the fate of complement in separate and particular diseases, a subject reserved for future papers, the following general conclusions may be drawn from this study of 278 cases of infectious disease.

1. While overall complement titer often changes in the course of infectious disease, the extent and changes are by no means always similar in different diseases, nor even in individual instances of the same disease.

2. Insofar as they were studied serially, 248 cases of infectious diseases showed a distribution of complement titers significantly different from that in a series of 40 normals; 30.6 per cent of the 248 patients exhibited complement titers less than 25 units per ml. of serum, the lower limit of "normal" titer. On the other hand, a minimum of 13.7 per cent of the patients showed titers over 50 units per ml. of serum, the upper limit of "normal" titer. In the control group of normal individuals, only 5.0 per cent showed over 50 units of complement. Since titers were not determined every day for a given patient, it is possible that additional low levels of titer were not observed,

so that the figure of 30.6 per cent must be taken as a minimum.

3. In 12 cases, or 4.3 per cent of a total of 278 patients, the complement titer was found to be as low or lower than 10 units per ml. of serum. The low titer usually coincided with a serious phase of the patient's condition; with the improvement of this condition the titer would tend to return to the normal value. However, of these 12 patients, 4 died, giving a fatality rate for this group of 33.3 per cent. This is a striking rate when compared with the figure of 5.0 per cent fatality for the entire group of 278 patients.

4. In those cases showing a decrease in overall complement titer there was a marked decrease or disappearance of C'4; in other cases, besides the diminution of C'4 titer, a decrease of C'2 and C'1 titers was encountered. C'3 titer did not diminish at all, except in one case in which it changed slightly. All of the cases exhibiting no complement titer whatsoever also showed no C'4 titer, a fact which is even more striking when it is recalled that this component is present in abundance (*i.e.*, its titer is considerably higher than the titers of C'2 and C'3) in normal human complement.

When diminished titers improved there was a concomitant increase in the titers of the diminished components.

5. Among the separate diseases, the occurrence of a low titer was most frequent in cases of epidemic (meningococcal) meningitis, in which 19 of 38 patients studied were shown to have diminished titers. Among patients with subacute bacterial endocarditis, 2 of 5 cases studied exhibited low titers.

6. High titers, *i.e.*, titers over·50 units per ml. of serum, were most frequently observed in cases of erysipelas. However, significant numbers of patients with scarlet fever and of those with pneumococcal infections showed increased titers. All 4 components of complement apparently participated in this increase.

7. As yet the authors can see no *general* prognostic value in complement titer, although it may be said that in those relatively few cases in which the titer diminished to very low levels, *e.g.*, 10 units per ml. of serum, the prognosis is grave. While experience has shown that patients with low titer survived in 2 out of 3 instances, it has also shown

that those in whom low titer *persists,* and particularly low C'4 titer, succumbed.

It must be further remarked that not all patients with infectious disease who succumbed exhibited low titers immediately preceding their death. As a matter of fact, 2 patients with pneumococcus Type III meningitis exhibited extraordinarily high titers of 86 units per ml. in the days preceding death.

The question of the prognostic value of complement titer, however, is not a closed issue.

8. Observing all of the precautions counseled in the main body of this paper, serum protein concentration appears to bear relationship to complement titer. Modified by more significant factors which determine titer, a low protein concentration disposes a serum toward low titer, and a high protein content toward high titer.

9. In general, there is no apparent relationship between a patient's total white blood cell count and complement titer. In cases of meningococcus meningitis, however, the coincidence of low titer and high cell count may be significant. Whether these are independent events in the course of this particular disease, or are related through the phenomena of opsonification and phagocytosis, is not known.

10. No apparent relationship between body temperature and complement titer was found.

11. These studies have been carried out over a period of several years, in which time some progress has been made in the titration of the complement complex. Stimulated by the fact that a more reliable method of complement titration is necessary to clarify the problem of complement in disease, such a method has been developed in this laboratory eliminating most of the errors found in previous methods, and will be the subject of a future paper.

12. Keeping in mind the dictum of Osborn quoted in the introduction to this paper, the present authors deem it premature to speculate on the full meaning of these changes of titer in infectious disease. Their future work will be aimed at determining the mechanisms of diminution or increase of titer in particular diseases, and the role of complement fixation, opsonification, anticomplementary substances, etc., in this respect.

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COMPLEMENT AND ISOHEMAGGLUTININS IN URINARY PROTEINS [1]

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It has been recently established that the diminution or extinction of the serum complement titer which sometimes occurs in the course of infectious disease is coincident with a decrease or disappearance of primarily $C'4$ and secondarily $C'2$ and $C'1$ (1). It was also found that prognosis was grave for those patients whose complement titers fell to zero or near-zero. The mechanism of masking, inactivation, or interference with the production of these components, whatever the case may be, has not as yet been fully investigated, nor has the manner in which the dimution of titer may contribute to greater susceptibility to infection.

In cases of kidney disease, in which sometimes a lowering of serum complement has also been observed, the excretion of protein in the urine presents a situation distinct from that generally found in cases of uncomplicated infectious disease. While not always reflecting itself in a decrease of serum complement titer, for reasons discussed below, a loss of complement by means of urinary excretion nevertheless could take place in kidney disease, and could be a contributory factor to decreased resistance to superimposed infection.

The present study is therefore concerned with the analysis, chiefly with respect to the complement components, of a series of urine specimens obtained from normal individuals, from cases of infectious disease, and from patients with kidney disease.

METHODS

1. Preparation of urine specimens. Urine samples, consisting of single voidings, were usually processed within a few hours after collection. Specimens were measured for volume, adjusted to pH of about 6.6 with either 0.1 N NaOH or 0.1 N HCl, filtered or centrifuged to remove insoluble material, and chilled to 1° C. One of 2 methods, both of which were designed to preserve complement activity and both of which gave comparable results, was then used to prepare urine or urinary protein solutions for testing. These methods are as follows.

(*a*) The urine, which had been given preliminary treatment as described above, was dialyzed at 1° C. for 24 hours against 2 changes of medium, each consisting of 4 liters of 0.9 per cent NaCl mixed with 20 ml. of potassium phosphate buffer of pH 6.6 and ionic strength 0.3. This method was used particularly for apparently highly concentrated urines excreted in low volumes.

(*b*) The urine, adjusted to pH 6.6 and filtered, was diluted with $\frac{1}{4}$ its volume of the potassium phosphate buffer of pH 6.6 and ionic strength of 0.3. Thirteen grams of ammonium sulfate were then added for each 25 ml. of the buffered urine, and the mixture shaken until all of the salt was dissolved. The resulting mixture was then allowed to stand at 1° C. for at least 1 hour, following which it was filtered just to dryness at 1° C. on a fine sintered glass funnel. The collected precipitate was then dissolved in a minimum quantity of the buffered saline prepared as described under Method (*a*), the protein solution enclosed in a cellophane membrane, and dialyzed against repeated changes of the buffered saline at 1° C. until free from sulfate. This latter process required from 24 to 36 hours. The contents of the dialysis sac were then centrifugalized at 1° C., the supernate was poured off, measured for volume, and tested for complement component activity. Occasionally the precipitate which formed upon dialysis was more than scant, but invariably it was insoluble in 18 per cent NaCl or phosphate buffer of pH 7.8. Suspensions of these precipitates, when tested for complement component activity, were always found to be negative.

This method led to a complete separation of the urinary proteins.

2. Testing for complement component activities. Testing complements, previously designated as specifically inactivated complements, were prepared from fresh human serum as described elsewhere (2). The dialyzed urine or the urinary protein solution, in amounts of 0.15, 0.30, and 0.50 ml., was tested alone and in combination with each of the various testing complements used in 3-unit amounts. After incubation for 30 minutes at 37.5° C. with the standard dose of sensitized sheep red cells, the test mixtures were centrifugalized and their supernates read for per cent hemolysis by visual comparison with prepared standards (2).

Since all of the dialyzed urines and the urinary proteins were inactive when tested alone, the degree of hemolysis which they produced in combination with an *excess* of a given testing complement was a measure of the amount of the given component present in the urine solution.

3. Detection of isohemagglutinins. In some cases the

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dialyzed urine or the urinary protein solution was tested by both slide and test tube methods for isohemagglutinin type against A, B, and O cell suspensions.

4. *Electrophoresis.* Several samples of treated urines were subjected to electrophoresis using a veronal buffer of pH 7.8 and ionic strength of 0.1.

RESULTS

1. Complementary activity

Table I summarizes the significant data obtained with respect to complement activity of the urinary proteins. In the table a given complement component activity is expressed in the number of units per gram of protein found in, or isolated from, the urine, which caused complete hemolysis of the standard dose of sensitized sheep red cells in the presence of an added excess of the other 3 components. As a basis for comparison, the table includes the number of such units of each of the components which would be found in a normal serum of average complement titer (33.3 units per ml. of serum) and protein content (7.0 per cent). Since the complement components

are associated with the globulins, their units could more properly be expressed per gram of *globulin*, but even such designation would be arbitrary, and accordingly is made only in 2 instances.

a. Normal urines. Urine specimens from 3 normal individuals were treated according to Method (*b*). In each case a very minute amount of protein was obtained which showed no complement component activities.

b. Infectious diseases. The urines of 2 patients with meningococcal meningitis, one with scarlet fever, and one with both rheumatic fever and Type XII pneumococcal meningitis, were studied. Although all of the urines contained some protein, no complement component activities were detected in any of them. The data obtained with respect to the rheumatic fever-meningitis patient, because of the relatively high urinary excretion of protein, are included in Table I.

c. Nephrotic syndrome; lipoid nephrosis. Urine specimens from 3 children were treated and tested; complement components were found in the urinary proteins of each case at one time or another

TABLE I

Content of complement components in urinary proteins

Patient	Diagnosis	Date of urine sample	Total protein in urine	Approximate number of units of C' component isolated from the urine			
				C'1	C'2	C'3	C'4
			grams per cent		*per gram protein*		
D. C.	Rheumatic fever; Type XII pneumococcal meningitis	3-3-45	0.44	0	0	Tr?	Tr?
D. M.	Lipoid nephrosis	2-5-45	0.58	100	440	100	100
D. M.	Lipoid nephrosis	2-13-45	0.32	0	80	80	80
A. S.	Lipoid nephrosis; upper respiratory infection	2-5-45	0.33	0	200	80	80
A. S.	Lipoid nephrosis; upper respiratory infection	2-13-45	0.56	0	40	0	20
J. G.	Lipoid nephrosis	2-5-45	0.42	40	250	200	100
J. Q.	Chronic glomerulonephritis; nephrotic syndrome; upper resp. infect.	4-21-44	0.54	0	425	0	850
L. B.	Acute glomerulonephritis	5-2-44	0.61	0	475	700	650
E. M.	Acute glomerulonephritis; nephrotic syndrome	2-27-45	1.73	0	40	90	80
M. P.	Arteriosclerotic heart disease; diabetes mellitus	2-22-45	0.22	0	250	175	70
			Total globulin in urine	Units C' component isolated from urine			
			grams per cent		*per gram globulin*		
D. M.	Lipoid nephrosis	2-13-45	0.08	0	275	275	275
E. M.	Acute glomerulonephritis; nephrotic syndrome	2-27-45	0.79	0	90	200	180
Units C' component per gram of protein in an average normal serum (approximate)				4,275 10,300	475 475	475 700	4,275* 11,200†

* Approximated as described by Ecker, E. E., Seifter, S., and Dozois, T. F., J. Lab. Clin. Med., 1945, **30**, 39.
† Approximated from figures given by Bier, O. G., Leyton, G., Mayer, M., and Heidelberger, M., J. Exper. Med., 1945, **81**, 449.

in the course of the disease, but 2 of the total of 7 urine samples failed to show any component activity whatsoever. As shown in Table I, C'2 and C'3 chiefly were excreted, followed by C'4, and in 2 of the 5 urine samples, by C'1.

All 3 of these patients were edematous.

d. Glomerulonephritis. One urine sample from each of 2 patients, and 2 samples from a third patient, were studied. C'2 and C'4 were present in all of the urines, C'3 was present in 3 of the samples, and C'1 was present in none.

These patients were also edematous.

e. Arteriosclerotic heart disease and diabetes mellitus. Two urine samples obtained from one patient were studied. One of these contained about 1 per cent protein, and showed considerable C'2 and C'3 activities and some C'4. The second sample contained relatively slight traces of protein, and was found to have no complement component.

This patient also was edematous.

f. Arteriolar nephrosclerosis. One urine obtained from a patient was examined and found to contain a very minute amount of protein with questionable traces of C'1, C'3, and C'4 activities.

This patient did not have edema.

2. Isohemagglutinin activity

As a beginning in the study of the excretion of antibodies, the isohemagglutinin activities of a number of dialyzed urines and the urinary protein solutions were determined qualitatively. After these tests were completed, the clinical records of the patients were checked for blood types, and in every case in which such a record was available it served to confirm the urinary tests if these were at all positive. In several cases, particularly in which the content of urinary protein was low, no tests were obtained at all. Table II gives a summary of the positive results obtained by test tube titration.

3. Electrophoresis

The urinary proteins obtained in one case with nephrotic syndrome and those from one case of acute glomerulonephritis were examined in the Tiselius apparatus. In confirmation of the observations of Luetscher (3) and Blackman and Davis (4), Table III shows that the urine of this patient contained considerably more albumin than

TABLE II

The isohemagglutinin activities of urinary proteins

Patient	Date	Clumping by urinary proteins of cells of type			Blood type from urine	Blood type from hospital records
		A	B	O		
D. M.	2-13-45	−	++++	−	A	A
D. M.	2-19-45	−	+++	−	A	A
A. S.	2-13-45	−	±	−	A?	A
A. S.	2-22-45	−	−	−		A
M. P.	2-20-45	+	++++	−	O	O
E. M.	2-27-45	−	++++	−	A	A

did that of the nephritic patient, and that the latter urine contained a large amount of gamma globulin. What is of particular interest, in relation to the present study, is that both of these urinary protein solutions contained complement activity and isohemagglutinins, and that the globulin fractions generally associated with these activities were shown to be present in the electrophoretic diagrams.

TABLE III

Electrophoretic analysis of urinary proteins

Patient	Diagnosis	Date	Concentrations (per cent of total)			
			Albumin	Globulins		
				Alpha	Beta	Gamma
D. M.	Nephrotic syndrome	2-13-45	74.5	2.6	9.8	13.0
E. M.	Acute glomerulo-nephritis; nephrotic syndrome	2-27-45	55.1	6.7	10.5	27.9

DISCUSSION

In the several samples of urine obtained from normal individuals and from people suffering with uncomplicated infectious disease, no complement component activities were detected. On the other hand, the data in Table I show that in one case or another each of the 4 complement components was identified in the urinary proteins excreted in kidney disease, particularly in those cases in which there was an associated edema. In this respect, the edema probably has no significance other than as an indication of the profound reduction of the serum proteins as evidenced by the following: DM had a serum protein content of 3.3; AS, 4.3; EM, 3.62; JG, 5.6; and JQ, 4.81 per cent.

As seen in Table I, the consistent and high excretion of C'2 is outstanding, the activity figures

often approaching that for the normal serum. In sharp contrast are the infrequency of C'1 excretion, and the relatively low value obtained for this component even when it is present. Since the excretion of a protein through the damaged kidney may be related to its solubility, it must be pointed out that C'2 is extremely soluble even in distilled water, whereas C'1 behaves as a euglobulin, and is easily precipitated. Furthermore, C'1 has an apparent isoelectric point at about pH 6 (5), and may be one of those more insoluble globulins, discussed by Luetscher (3), which are precipitated from dilute solutions of pH 5.5 to 6.5, and aid in the formation of the urinary "casts." Blackman and Davis (4) believe that the hyaline materials which collect in the glomeruli and tubules in patients with "progressing nephrotic nephritis," are probably derived from globulins other than fibrinogen. It may be, then, that C'1 is one of these globulins, and is retained in the process of formation of the hyaline materials.

The excretion of C'3 and C'4, both of which are in themselves relatively soluble, or at least may be attached to soluble proteins, is fairly consistent, though not as striking in amount as is the excretion of C'2.

Though red blood cells were found in some of the urine specimens tested, particularly in 2 of the cases of glomerulonephritis, the occurrence of complement components cannot be attributed in any great measure to the presence of whole blood, for the following reasons: (a) In a number of urine specimens in which complement components were present, no red blood cells were seen nor were positive benzidine tests obtained. (b) Contrary to what would be expected if whole blood were present, the entire complement complex was not found, but only certain components. (c) The amounts of complement components, particularly of C'2 and C'3, found in those urines showing some red cells, were relatively so great, that the urines would have had to consist in large measure of whole blood, a circumstance ruled out by the relatively low red cell and protein contents of the urines. From a physiologic standpoint, the discovery of complement components in the urine is extremely interesting, inasmuch as it demonstrates that normal plasma proteins, and particularly those with specific functions, are excreted in kidney disease. In addition to the complement compo-

nents, the isohemagglutinins have been demonstrated in the urinary protein excreted in kidney disease. While the authors have not as yet had the opportunity to study the excretion of antibodies to infectious agents, there is no reason to believe that these are not excreted.

The possible immunological significance of this work now becomes apparent. It is well known that a patient with kidney disease is particularly subject to infection, frequently pneumococcal in origin, and expressing itself as a septicemia, peritonitis, or an upper respiratory disease. This general lack of resistance to infection exhibited by individuals with kidney disease may in part be related to excretion of protein, as follows: (a) in the sense of Cannon (6), by the depletion of protein reserves, with consequent reduction of matrix necessary for the production of antibodies and complement; (b) by the loss to the urine of preformed antibodies, as suggested by Bell (7); (c) by the loss over a period of time of complement components, particularly C'2 and C'4, which together with C'1 are necessary for the acceleration of opsonification of invading organisms (8); and (d) by the diminution in the serum of any one or more of the 4 components of complement, all of which are necessary for bactericidal action (9).

If sufficient complement is excreted in the urine to predispose the patient, in some degree, to infection, a concomitant reduction of the serum complement should be noted. In some cases of kidney disease a simultaneous lowering of the serum complement titer and serum protein content has been observed; however, this has not been the general rule. The usual test for serum complement titer, being a test for a *dissolved* protein complex and therefore dependent upon *plasma volume*, necessarily fails to reveal the full character of changes occurring in the plasma. Plasma volume variations taking place with edema might tend to offset and mask the decrease of serum protein. Furthermore, the patients studied here have been undergoing treatment, inclusive of transfusions of whole blood and plasma, thus complicating even more the expected relationship between loss of serum protein to the urine and decrease of serum complement titer. These considerations are the basis for the statement made at the beginning of this paper that loss of serum protein may not always be reflected in diminished complement titer.

SUMMARY

1. Complement component activities have not been detected in, or isolated from, the urines of 3 normal individuals, nor in those of 4 people suffering with infectious disease.

2. Complement component activities have been identified in the precipitated urinary proteins of patients showing nephrotic syndrome, and in cases of acute and chronic glomerulonephritis, as well as arteriosclerotic heart disease with a nephrotic component.

3. The excretion of C'2 was most consistent and in largest relative quantity. C'3 and C'4 were excreted in the urines of these cases with good consistency, but C'1 was seldom excreted, and then only in relatively small amount.

4. It is pointed out that C'2 is a very soluble protein, whereas C'1 is a euglobulin; and that the high excretion of the one and the retention of the other is probably related to their solubilities.

5. Isohemagglutinins have been isolated with the urinary proteins in 5 instances. Identification of these was in agreement with the blood types of the patients.

6. The physiological significance of these findings lies in the demonstration that normal plasma proteins, and particularly those with specific functions, may be excreted in the urine by patients with kidney disease.

7. The immunological significance of this study is that it may explain in part the predisposition of patients with kidney disease to infection. The loss of complement substances and antibodies, both necessary for the opsonification and killing of invading organisms, may be contributing factors to the diminished resistance of these cases.

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ELECTROPHORETIC ISOLATION OF A CIRCULATING ANTICOAGULANT

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We recently reported (1) on the properties of a circulating anticoagulant found in the blood of a hemophiliac. At that time it was shown that the anticoagulant activity was associated with the globulin fractions of the plasma. In this paper we wish to report on the electrophoretic fractionation of plasma from this patient, and to present data indicating that the anticoagulant activity is associated with the γ globulin.

METHODS AND MATERIAL

The plasma used in these studies was obtained in one bleeding collected into one-ninth of its volume of 0.1 M sodium oxalate. The blood was then centrifuged and the plasma stored at −20° C. until used. There was no decrease in the anticoagulant activity under these conditions.

The electrophoretic fractionations were carried out in a large cell, with a capacity of 100 ml., similar to that employed by Blix, Tiselius and Svensson (2), consisting of 4 center sections each 46 mm. high with a cross-section of 50 × 7.5 mm. Since the fractions obtained were to be tested for their anticoagulant activity, a barbiturate buffer of pH 7.5 was employed. The buffer contained 0.01 M sodium diethylbarbiturate, 0.006 M hydrochloric acid and 0.15 M sodium chloride. The undiluted plasma was dialyzed in 18/32 inch Visking tubing for 24 hours against 2 changes of 1,000 ml. of buffer, and for 24 hours against 2,000 ml. of the buffer. As suggested by Tiselius (3) the lower half of each electrode vessel was filled with a buffer having 4 times the concentration of the buffer against which the plasma was dialyzed. The remainder of the electrode vessels and the cell were filled with the buffer employed for the final dialysis. Electrophoresis was carried out for 67 hours at 1° C. with a constant potential gradient of approximately 1.5 volts per cm.

The separated fractions were removed through a long capillary tube attached to a 50 ml. syringe. The material was withdrawn at a constant rate of approximately 0.5 ml. per minute. This was done by attaching the plunger of the syringe to a screw drive, which was driven by a synchronous motor through a suitable gear train. The tip of the capillary was lowered to the desired position by means of a screw moved by 2 spiral gears. Under schlieren observation 2 fractions were removed from each side; namely, (1) ascending albumin plus α globulin, (2) ascending albumin plus α and β globulins, (3) descending γ globulin plus fibrinogen, and (4) descending γ and β globulins plus fibrinogen.

The fractions were tested for their anticoagulant activity by determining the extent to which they would prolong the coagulation time of recalcified normal plasma. Previous studies (1) have shown that 0.2 ml. of the plasma containing the anticoagulant will markedly prolong the coagulation time of 0.4 ml. of normal plasma. In testing the fractions obtained by electrophoresis a similar procedure was used.

No attempt was made to remove the buffer by dialysis before testing the samples, since it was found that the buffer used in this study did not affect the coagulation time of recalcified normal plasma to any greater extent than an equal volume of 0.15 M sodium chloride.

Besides the various fractions, the unfractionated material remaining in the bottom section of the cell was tested. This gave an indication of whether any changes had occurred in the anticoagulant activity during electrophoresis.

The protein content of the fractions was determined by digesting a suitable aliquot with sulfuric acid and superoxol followed by Nesslerization. The color developed was read in the Evelyn colorimeter. A correction was applied to the nitrogen values so obtained for the nitrogen contained in the buffer, following which the protein was calculated by multiplying by the factor 6.25.

RESULTS

The data obtained in this study are shown in Tables I and II. From Table I, showing the results of tests made on fractions obtained by electrophoresis in the large cell, it can be seen that only the fractions containing γ globulin displayed anticoagulant activity. Both the γ globulin plus fibrinogen fraction and the γ and β globulins plus fibrinogen fraction showed anticoagulant activity.

TABLE I

Fractionation of whole plasma

Fraction added to normal plasma	Coagulation time* *min.*
Plasma after dialysis	40
Albumin plus α globulin	3
Albumin plus α and β globulins	3
γ globulin plus fibrinogen	22
γ and β globulins plus fibrinogen	20
Plasma from bottom section of cell	44
Buffer	2½

* Each test was made using 0.2 ml. of the fraction, 0.4 ml. of normal plasma, and 0.4 ml. of 0.025 M calcium chloride.

TABLE II

Separation of γ and β globulin

Fraction added to normal plasma	Coagu-lation time*	Protein
	min.	*grams per 100 ml.*
γ and β globulins after dialysis	34	0.85
β globulin	4	0.25
γ globulin	24	0.39
Material from bottom section of cell	23	
Buffer	3	

* Each test was made using 0.2 ml. of the fraction, 0.4 ml. of normal plasma, and 0.4 ml. of 0.025 M calcium chloride.

The 2 fractions, albumin plus α globulin, and albumin plus α and β globulins, failed to show any anticoagulant activity.

The 2 fractions, γ globulin plus fibrinogen and γ and β globulins plus fibrinogen, obtained in the large cell fractionation, were mixed and the fibrinogen converted to fibrin by the addition of 1/20 volume of thrombin. The solution remaining after removal of the fibrin presumably contained only γ and β globulins.

This solution was fractionated in the 11 ml. divided cell (4) in such a manner that the upper half of the descending side contained γ globulin, and the upper half of the ascending side contained β globulin. Tests on these fractions (Table II) confirmed the observation previously made, that the anticoagulant under investigation migrates as a γ globulin.

SUMMARY

Plasma from a hemophiliac, containing an anomalous anticoagulant, has been fractionated by electrophoresis. Tests on the various fractions show that the anticoagulant activity is associated with the γ globulin fraction.

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A COMPARISON OF THE CEPHALIN–CHOLESTEROL FLOCCULATION AND THYMOL TURBIDITY TESTS IN PATIENTS WITH EXPERIMENTALLY INDUCED INFECTIOUS HEPATITIS [1]

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The cephalin-cholesterol flocculation test (1, 2) and, more recently, the thymol turbidity test (3) have been widely used to determine the presence and degree of hepatocellular dysfunction in various diseases of the liver. The former is believed to depend on a qualitative change of the serum albumin, as well as a quantitative alteration in serum globulin (4). The latter is thought to be concerned with the formation of a globulin-thymol-lipid complex (2, 3). In a recent comparison of the results of these tests it has been suggested (5) that there may be an essential difference between their mechanisms, so that they cannot be used interchangeably. It is apparent that when strongly positive, both tests may be indicative of hepatocellular damage. Thus, both are equally useful in infectious hepatitis when the disease is well established, and during early convalescence. However, there are no serial data available on the comparative appearance times of positive tests, or their subsequent return to normal during the course of this disease. On the basis of the prior appearance of a positive cephalin-cholesterol flocculation test in 2 cases of subacute yellow atrophy, it has been suggested that this might be true of epidemic infectious hepatitis (5).

During the past 18 months, experiments conducted by the Neurotropic Virus Disease Commission on the transmission of infectious hepatitis to human volunteers have made it possible to study certain tests of liver function in such patients (6). It is the purpose of this paper to report on the results of the Hanger and the Maclagan tests in 27 patients during the incubation period and course of experimentally induced infectious hepatitis.

METHODS AND MATERIALS

Subjects. The subjects were previously healthy, male human volunteers, ranging in age from 19 to 29 years. These men contracted infectious hepatitis experimentally following inoculation or ingestion of material known to contain the etiologic agent of the disease. The diagnosis of infectious hepatitis was made on the basis of characteristic symptoms and signs accompanied by fever and consistent deflection of the bromsulphalein dye [2] (7) retention and cephalin-cholesterol flocculation (2) tests. All patients in this report had clinical jaundice.

Virus. The strain of virus used in this laboratory was originally obtained from the stool of a U. S. Army soldier (BE) who contracted *epidemic infectious hepatitis* in Sicily in 1943 (8). It has been through 4 passages in human volunteers to date. This agent is filtrable through an L2 Chamberland filter, and withstands heating to 56° C. for at least 30 minutes (9). It has produced the disease in 27 out of 40 human volunteers (including those reported here) following parenteral or oral inoculation with incubation periods ranging from 15 to 34 days.

Laboratory observations. Four hundred cephalin-cholesterol flocculation tests, and 400 thymol turbidity tests were done on the sera of the 27 human volunteers. In practically all instances both tests were done on the same specimen of serum. Cephalin-cholesterol flocculation tests were performed according to the method of Hanger (2). Two different antigens [3] were freshly prepared on each day of test, and duplicate tests were done on each serum. The maximum amount of flocculation regarded as normal was a trace in 24 hours, and 1 plus in 48 hours. All tests were done within 2 hours of obtaining serum from patients. When the 2 antigens did not agree in sensitivity, the test showing the lesser amount of flocculation was recorded. Thymol turbidity tests were done according to the method of Maclagan (3). The maximum normal turbidity was regarded as 1 to 4 units, as measured against the formazin standards [4] of Kingsbury *et al* (10). Most of these thymol turbidity tests were performed on freshly drawn sera, but a certain number were done on sera which had been frozen and stored at dry ice box temperature for periods ranging from 2 to 12 months. It had been previously determined

[2] Retention of 10 per cent of dye 30 minutes after the intravenous injection of 5 mgm. of bromsulfalein per kgm. of body weight was considered the maximum normal.

[3] Obtained from Wilson and Co., Chicago, Ill.

[4] Standard Reagents Co., Philadelphia, Pa.

that sera stored in such manner suffered no apparent change in their degree of reaction with the thymol reagent.

In the experiments to be reported here, the cephalin-cholesterol flocculation and thymol turbidity were determined at least twice in each subject before experimental inoculation. All were normal. After inoculation of the subjects with hepatitis virus, tests were done every 7 to 10 days until the onset of disease, when they were performed twice during the first week, and then every 10 days for the ensuing 2 months. During the 3rd month after onset each man was tested at least once.

RESULTS

When cephalin-cholesterol flocculation and thymol turbidity tests were performed this frequently, a regular pattern of response in infectious hepatitis was observed. Both tests became positive in a certain number of patients during the acute febrile phase of disease. The 27 patients reported here may be almost equally divided into 2 groups. One group had an acute onset of disease with fever. The other group had an insidious onset with vague abdominal symptoms for periods of 2 to 11 days before the appearance of fever. In general, it was observed that the 13 patients who made up the former group tended to develop positive cephalin-cholesterol flocculation and thymol turbidity tests during the first week (febrile period) of disease. In contrast, it was evident that the 14 patients in the latter group who had a vague onset with mild generalized abdominal complaints and a normal temperature, usually did not develop alteration in these tests until several days later after the appearance of fever.

By using the appearance of fever as a fixed point, it is possible to present the combined changes occurring in cephalin-cholesterol flocculation and thymol turbidity in all 27 patients of this series. When this is done, and the average duration of fever and jaundice and the appearance time of jaundice are recorded, it is seen that positive cephalin-cholesterol flocculation and thymol turbidity tests occur in a certain proportion of patients during the febrile phase before the appearance of jaundice, and that with defervescence and appearance of jaundice at the end of the 1st week of disease almost all patients tested had positive responses.

For purposes of convenience the results of cephalin-cholesterol flocculation and thymol turbidity tests are described as occurring in 4

phases; namely (1) the incubation (pre-febrile) phase, (2) the febrile (pre-icteric) phase, (3) the icteric (post-febrile) phase, and (4) the convalescent (post-icteric) phase.

Incubation (pre-febrile) phase.

When incubation periods were measured from the day of inoculation to the first appearance of symptoms they ranged from 15 to 28 days. However, the time elapsed from day of inoculation to onset of fever in these patients ranged from 24 to 30 days. Using the onset of fever as a fixed point, the results of the thymol turbidity and cephalin-cholesterol flocculation are recorded for 4 weeks before fever, and 3 months after fever, in 27 patients (Figure 1).

Thymol turbidity tests all remained normal throughout the incubation period, with the exception of one patient who had a single determination of 5 units 4 days before the onset of fever, when he was asymptomatic and when all other tests of liver function including the cephalin-cholesterol flocculation were normal.

The cephalin-cholesterol flocculations were normal during this period, with the exception of positive tests in 2 patients 1 and 3 days respectively before the onset of fever. Both of these patients were having gastrointestinal symptoms at the time, but all other tests of liver function were normal.

Febrile (pre-icteric) phase.

The duration of fever ranged from 4 to 14 days, averaging 8 days. Eighteen of the 27 patients had positive cephalin-cholesterol flocculation at some time during their febrile phase. During the first 2 days of fever most of the cephalin-cholesterol flocculation tests recorded were normal. From the 3rd day on, however, the preponderance of tests were positive.

Ten patients had positive thymol turbidity tests in their febrile phase. During the first 4 days of fever most of the thymol turbidity tests recorded were normal. The number of positive tests increased on the 5th day, and on the 6th and 7th days a preponderance of the tests were positive.

It is evident that the cephalin-cholesterol flocculation became positive significantly earlier than the thymol turbidity test. In Table I are recorded the results of these 2 tests during the febrile pe-
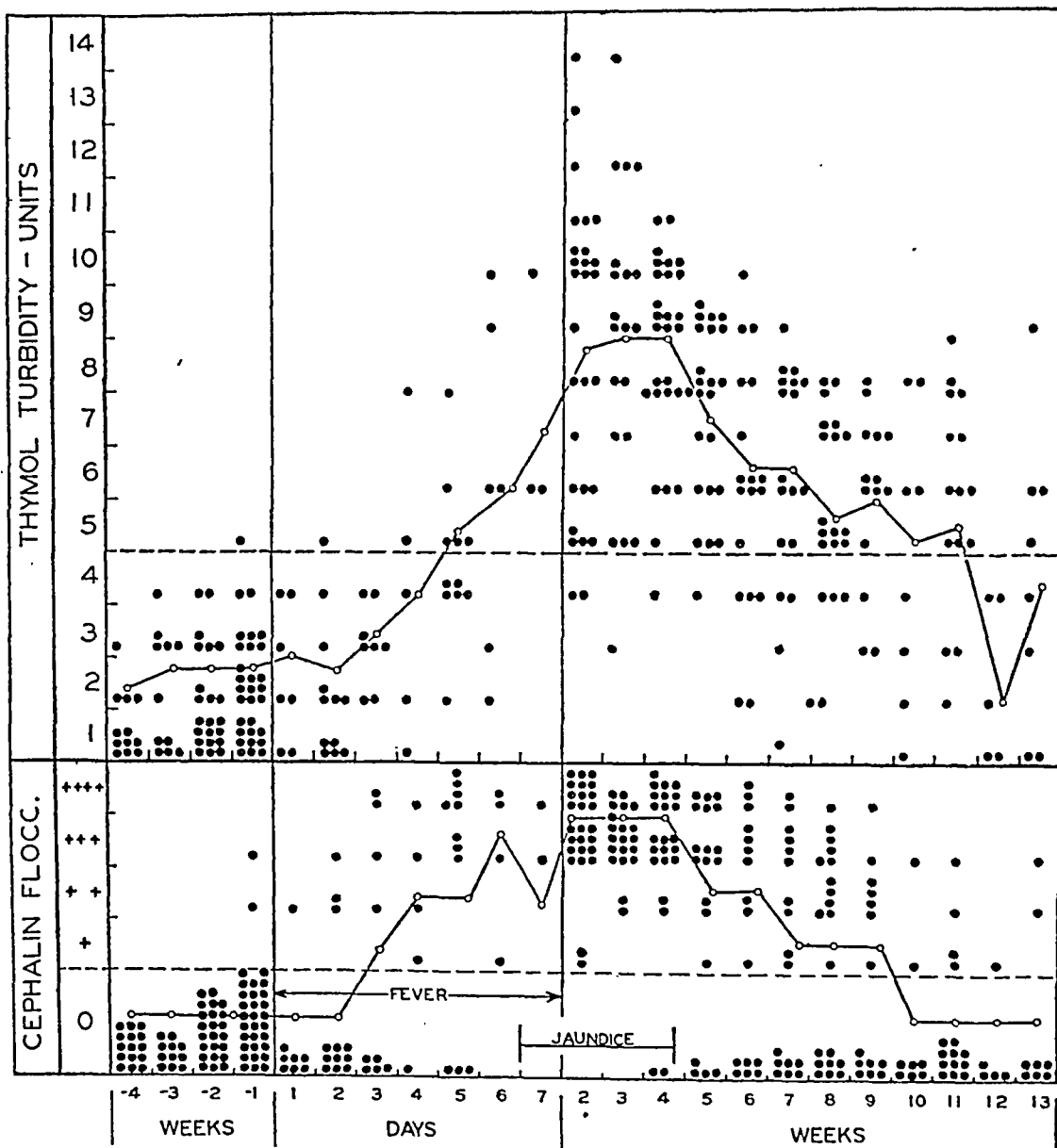
FIG. 1. CEPHALIN-CHOLESTEROL FLOCCULATION AND THYMOL TURBIDITY TESTS IN 27 PA-
TIENTS DURING THE INCUBATION PERIOD, COURSE OF DISEASE, AND CONVALESCENCE OF EXPERI-
MENTALLY INDUCED INFECTIOUS HEPATITIS

(—) indicates weeks before the onset of fever. Black dots indicate individual determinations which are expressed as an average by the unbroken lines. The duration of fever and jaundice and the appearance time of jaundice represent the averaged experiences of the 27 patients. The horizontal (broken) lines indicate the normal maximum level of cephalin-cholesterol floccula-tion and thymol turbidity tests.

riod. In the first 5 days of fever before the ap-pearance of jaundice, 20 out of 45 sera had posi-tive cephalin-cholesterol flocculations, while only 8 out of 43 of these sera had positive thymol tur-bidity tests. Attention is called to the fact that a certain number of patients had their 2 sets of tests within the first 4 days of fever. In view of the greatly increased percentage of positive tests in

the last 3 days of the febrile period, it is quite pos-sible that had all patients been tested at this time a greater number might have been positive.

Icteric (post-febrile) phase.

Clinical jaundice ranged in appearance from 3 to 12 days after the beginning of fever, averaging 6 days. The duration of jaundice ranged from 8

TABLE I

Cephalin-cholesterol flocculation and thymol turbidity tests in patients with experimentally induced infectious hepatitis

Day of fever	Cephalin flocculation		Thymol turbidity	
	Tested	Positive	Tested	Positive
	number of sera			
1	8	1	7	0
2	12	3	12	1
3	9	4	8	0
4	5	4	5	2
5	11	8	11	5
6	4	4	6	4
7	2	2	3	3
8	6	6	8	7
Total	57	32	60	22

to 31 days, with an average of 20 days. Characteristically clinical jaundice appears with defervescence at the end of the first week. The cephalin-cholesterol flocculation and thymol turbidity tests were positive at some time during the icteric phase of all patients, with the exception of one who was mildly sick and failed to develop positive thymol turbidity, although other tests of liver function were positive. During the 2nd week of disease, as clinical jaundice increased, the cephalin-cholesterol flocculation and thymol turbidity tests with rare exceptions were strongly positive. This continued throughout the period of jaundice, through the 4th week of disease.

Convalescent (post-icteric) phase.

During the 5th week of disease the cephalin-cholesterol flocculation remained positive in general, and it is not until the 6th week that the number of negative tests increased materially. During the 7th, 8th, and 9th weeks of disease the cephalin-cholesterol flocculation became less positive in many patients, and the number of negatives increased so that by the 10th week the average of tests was within normal range, although from there through the 13th week an occasional patient had a positive test. During the period of observation, 20 patients had a return to negative cephalin-cholesterol flocculation. The duration of positive tests in these patients ranged from 25 to 87 days, with an average of 53 days. Seven patients still had mildly positive tests at the last time of observation, ranging from 77 to 172 days after onset of disease.

From the 5th week of disease the degree of positivity of the thymol turbidity tests was less

TABLE II

Disagreement in results of cephalin-cholesterol flocculation and thymol turbidity tests in 23 patients with experimentally induced infectious hepatitis

Week after fever	Thymol turbidity	Cephalin-cholesterol flocculation	
	units	24 hours	48 hours
−1	5	0	0
	2	++	+++
	2	+++	+++
1	4	++	+++
	2	0	++
	2	+++	++++
	3	++	+++
	3	++++	++++
	4	++++	++++
	2	+++	++++
	4	++	+++
	1	+	++
	2	+++	++++
	6	0	±
	4	+++	++++
	4	++++	++++
	4	++++	++++
	4	++++	++++
	3	±	++
2	4	++++	++++
	4	++	++
3	3	++	+++
4	6	0	0
5	9	0	0
	9	0	+
	5	0	0
6	4	+	++
	8	0	0
	6	0	0
7	8	0	0
	5	0	+
	8	0	0
8	5	0	0
	5	0	0
	7	0	0
9	6	0	+
	8	0	0
	7	0	0
	7	0	0
	6	0	0
10	6	0	0
	8	0	0
	4	+	++
	6	0	0
11	6	0	0
	8	0	0
	6	0	0
	6	0	0
	5	0	0
	8	0	0
	5	0	0
12	4	+	++
13	5	0	0
	6	0	0

(−) before fever

strong, and a gradually increasing number of patients fell within the normal range, so that by the 12th week the average determination of thymol turbidity was normal, although several patients continued to have positive tests. Only 14 of 26 patients had a return to normal thymol turbidity during the period of observation. The duration of positive tests in these patients ranged from 30 to 112 days, with an average of 64 days. Twelve patients still had mildly positive tests at the last time of observation, ranging from 75 to 172 days.

Agreement of tests. In 23 of the 27 patients with experimentally induced infectious hepatitis there was qualitative lack of agreement in the results of the cephalin-cholesterol flocculation and thymol turbidity tests at some time during the period of observation, extending from the day of inoculation through convalescence. This occurred 60 times in a total of 360 simultaneous tests (16 per cent). In Table II are recorded the results of these tests for 13 weeks. The preponderance of early disagreement occurred in the first 5 days of the febrile, and was dependent on the fact that the cephalin-cholesterol flocculation became positive earlier than the thymol turbidity test. From the end of the first week until the middle of the 4th week there was almost complete agreement of tests qualitatively, with the exception of rare negative thymol turbidity in the presence of positive cephalin-cholesterol flocculation. After the 4th week, however, there appeared a gradually increasing number of tests which failed to agree in positivity. In contrast, however, the failure of agreement at this time was dependent on the persistence of positive thymol turbidity tests in patients whose cephalin-cholesterol flocculations had returned to normal. Wide quantitative variation in degree of positivity in the 2 tests occurred, and there appeared to be little correlation between them except in the active early icteric phase of disease, when both tests were strongly positive in almost all patients.

DISCUSSION

It has been pointed out in this paper that the cephalin-cholesterol flocculation test becomes positive at a significantly earlier period than the thymol turbidity test in experimentally induced infectious hepatitis. In agreement with the results of others, both tests are of equal value when

the disease is well established, and at this time there is almost complete agreement in positivity between them both qualitatively and quantitatively. However, early in disease in the pre-icteric period, and late in the convalescent period, disagreement in positivity occurs in a regular pattern. This is dependent on the fact that the cephalin-cholesterol flocculation becomes positive earlier than the thymol turbidity and returns to normal before the latter test. The fact that this type of disagreement occurred in 23 out of 27 patients at some time during the course of their infectious hepatitis lends support to the suggestion of other investigators (5, 11) that the mechanism of the 2 tests may not be the same, and therefore they cannot be used interchangeably. The significantly greater proportion of positive cephalin-cholesterol flocculation tests in comparison with thymol turbidity tests in the first 5 days of the febrile phase of infectious hepatitis makes the former test of greater value in early diagnosis. At present, it is difficult to interpret the prognostic significance of the fact that the thymol turbidity remains positive longer in convalescence than the cephalin-cholesterol flocculation. It is not known whether this may represent activity of hepatitis or the persistence of some biologic change upon which the mechanism of the test is dependent (12).

SUMMARY

1. The results of serial determinations of the cephalin-cholesterol flocculation and thymol turbidity tests in patients during the incubation period and course of disease of *experimentally induced infectious hepatitis* are presented.

2. With rare exceptions both tests were negative during the incubation period, but became positive in all patients at some time during the course of disease, with the exception of one man with mild illness who had a normal thymol turbidity in the presence of other positive evidence of liver dysfunction.

3. Although both tests were equally positive in the active icteric phase of disease, the cephalin-cholesterol flocculation became positive earlier than the thymol turbidity test, and also returned to normal before the latter test.

4. The disagreement in positivity between the cephalin-cholesterol flocculation test and the thy-

mol turbidity test early in the pre-icteric phase and late in the convalescent phase lends support to the concept that their mechanisms may be different.

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METABOLIC STUDIES IN LOUSE–BORNE TYPHUS. OBSERVATIONS ON SERUM ELECTROLYTE PATTERN, SERUM PROTEIN PARTITION, AND NITROGEN BALANCE

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Although a great deal has been written about the clinical aspects of louse-borne typhus, there have been only a few reports on the metabolic changes which occur in this disease. Among the first biochemical studies on typhus were those reported by Murchison (1) in 1862. He discovered that large amounts of urea frequently were present in the blood of typhus patients, and that their urine contained abnormally large amounts of nitrogen. He attributed this to an "exaggerated disintegration of nitrogenous tissues." Since then other physicians have postulated excessive protein destruction in association with elevated blood and urine nitrogen (2, 3).

Renewed interest in this problem was aroused by recent studies (4) which showed that azotemia, or nitrogen retention, was a common finding in louse-borne typhus, and in general related to the severity of the case. In these studies it was reported that azotemia was present in every fatal case.

Little is known about the general electrolyte pattern in typhus. Murchison (1) noted that the chlorides were greatly reduced in the urine. He believed that this was due either to an impairment of absorption of chlorides or to their absolute retention. Since then other investigators have reported low chlorides in the blood of typhus patients (5). Julliard and Henaff (6) reported the serum and urine chlorides low until 1 week after defervescence. Woodward and Bland (3) reported the serum chlorides low and the carbon dioxide combining power high. They believed that a state of alkalosis existed in several of the patients, and suggested the use of ammonium chloride in the therapy. These workers also reported that often there was a reversal in the albumin-globulin ratio.

It appeared that a better knowledge of the electrolytes in this disease was essential for good supportive treatment. Consequently, studies were carried out on the salt intake and output and on the more prominent acid and basic radicals in the serum, including the proteins.

Because of the great protein destruction, it seemed important to know how much nitrogen was being excreted in the urine and what bearing it could have on the azotemia. It also seemed important to spare by dietary measures as much of the body protein as possible, therefore, studies were conducted along these lines.

Selection of patients. The patients in this study were unvaccinated Egyptian males between the ages of 12 and 48 years. The majority of these patients were thin and some of them were undernourished, but in no instances was avitaminosis observed. They were admitted to the United States of American Typhus Commission (7) ward in 1944–45 during the first 2 weeks of their disease. The diagnosis of louse-borne typhus was confirmed in all of these cases by the Weil-Felix and complement-fixation tests.[3] When patients received para-aminobenzoic acid (8) it is noted in the tables.

Estimation of severity of illness. After discharge from the hospital, each patient was classified according to the severity of his illness. This classification was based on the severity of the signs and symptoms, the duration of the disease and the occurrence of complicating conditions. With these factors in mind the following classifications were made:

"B"—Cases with minimal signs and symptoms, yet definitely diagnosed as typhus on clinical evidence.
"C"—Cases of moderate severity.
"D"—Severe cases with pronounced prostration.
"E"—Cases so severe that a fatal outcome was suspected at some point in the disease.
"F"—Fatal cases.

Methods. For the determination of carbon dioxide, chlorides, pH and total base, venous blood was collected

in oiled syringes without stasis, and placed under oil in centrifuge tubes. As soon as the blood clotted the tubes were stoppered and the excess oil removed. The serum was separated by centrifuging and transferred without delay to sampling bulbs containing mercury.

The carbon dioxide content of the serum was determined in a manometric Van Slyke apparatus. The chlorides were determined by a micro-modification of the open Carius method (9) and the pH in a Beckman pH meter. Analyses for total base were carried out by the benzidine method (9). The blood non-protein nitrogen was determined on oxalated specimens by the Nesslerization method (9).

The serum proteins were determined by the macro-Kjeldahl method (9).

The urine and feces were analyzed for nitrogen by the macro-Kjeldahl method. Twenty-four urine specimens preserved with thymol were analyzed daily. The feces were collected in glass jars containing sulfuric acid as a preservative. Combined collections over a period of 5 to 7 days were thoroughly mixed with water and analyzed. The daily fecal nitrogen was determined by dividing the total fecal nitrogen by the number of days. All analyses were performed in duplicate, and were repeated if the duplicates did not check.

Diets. Liquid diets were given to all of the patients. Some of the patients received a low protein, low caloric diet, while others received a high protein, high caloric diet. The low protein, low caloric diet was prepared to approximate the diet of the patients on the 3rd class hospital wards. A day's intake on this diet contained 6 grams of nitrogen, 1,800 calories and added salt. It was prepared with evaporated milk, corn syrup, sodium chloride and orange juice. The daily high protein, high caloric diet contained 3,400 calories, 21 grams of nitrogen and sodium chloride. It was prepared in a Waring blender with 1 liter of evaporated milk, 10 eggs, 35 grams of casein, 450 ml. of corn syrup, salt, water and flavoring. These diets were prepared under the supervision of a medical officer, and were analyzed frequently for nitrogen by the macro-Kjeldahl method. The "salt-free" diet was a low protein diet without added salt. It contained approximately 0.8 gram of sodium chloride, the amount of salt naturally occurring in 550 ml. of evaporated milk, and 500 ml. of orange juice. If the patient vomited he was excluded from the study.

ELECTROLYTE PATTERN

Results. Analyses of the carbon dioxide content and the chlorides of the serum in 34 cases revealed frequent abnormalities. In the first 2 weeks of disease the serum chlorides were low in 62 per cent of the cases, but only in 4 cases was the serum carbon dioxide content appreciably low. These results are shown in Tables I and II. There were no abnormally high chloride values except when the patients ingested sodium chloride, and only in one instance, Case 1031, was the serum carbon dioxide content elevated.

The pH was determined on most of the serum specimens concurrently with the chlorides and carbon dioxide. It was normal in all of the cases except patient 1098, who developed renal failure with marked azotemia and died on the 12th day of disease. On the day of death the serum pH was 7.20. At that time the non-protein nitrogen was 175 mgm. per cent, the carbon dioxide content

TABLE I

Data on the chlorides and carbon dioxide content of the serum in patients on "salt free" diets *

Case no.	Body weight	Day of disease	Serum Cl	Serum CO₂	Day of disease	Serum Cl	Serum CO₂	Day of disease	Serum Cl	Serum CO₂	Day of disease	Serum Cl	Serum CO₂	Days of fever	Severity of illness
	kgm.		*m. eq. per l.*	*m. eq. per l.*		*m. eq. per l.*	*m. eq. per l.*		*m. eq. per l.*	*m. eq. per l.*		*m. eq. per l.*	*m. eq. per l.*		
746	41.7	10	93	25	13	95	26	19	93	25				17	C
777	35.0	9	86	26	14	94	25							14	C
876	58.5	8	91	22										14	C
969	54.5	7	82	16										8	F
967	51.5	9	92	23	11	94	22	15	100	22				14	C
1020	59.8	9	84	26	14	102	28	17	109	23				18	C
1031	60.5	11	76	34	14	90	31	19	100	28				12	B
1098	56.8	7	93	23										12	F
1562	46.0	7	97	25	11	96	24	14	102	28				13	D
3955	62.8	7	94	26										10	F
5225	56.8	8	91	22	12	88	22	16	96	21				12	E
4441	51.0	8	95	24	10	96	26	12	89	25	13	80	24	25	D
1347	56.8	8	95	23	11	87	24	14	84	22	16	91	25	15	D
1285	55.0	14	91	25										26	D
1584	47.0	13	98	25	16	95	23							15	D
4749	50.0	10	84	28										17	C
4854	39.6	10	82	16	13	87	16	15	86	28				16	F
4934	48.2	8	82	25										9	F

* The salt content of a day's supply of the salt free diet contained approximately 0.8 grams of sodium chloride.
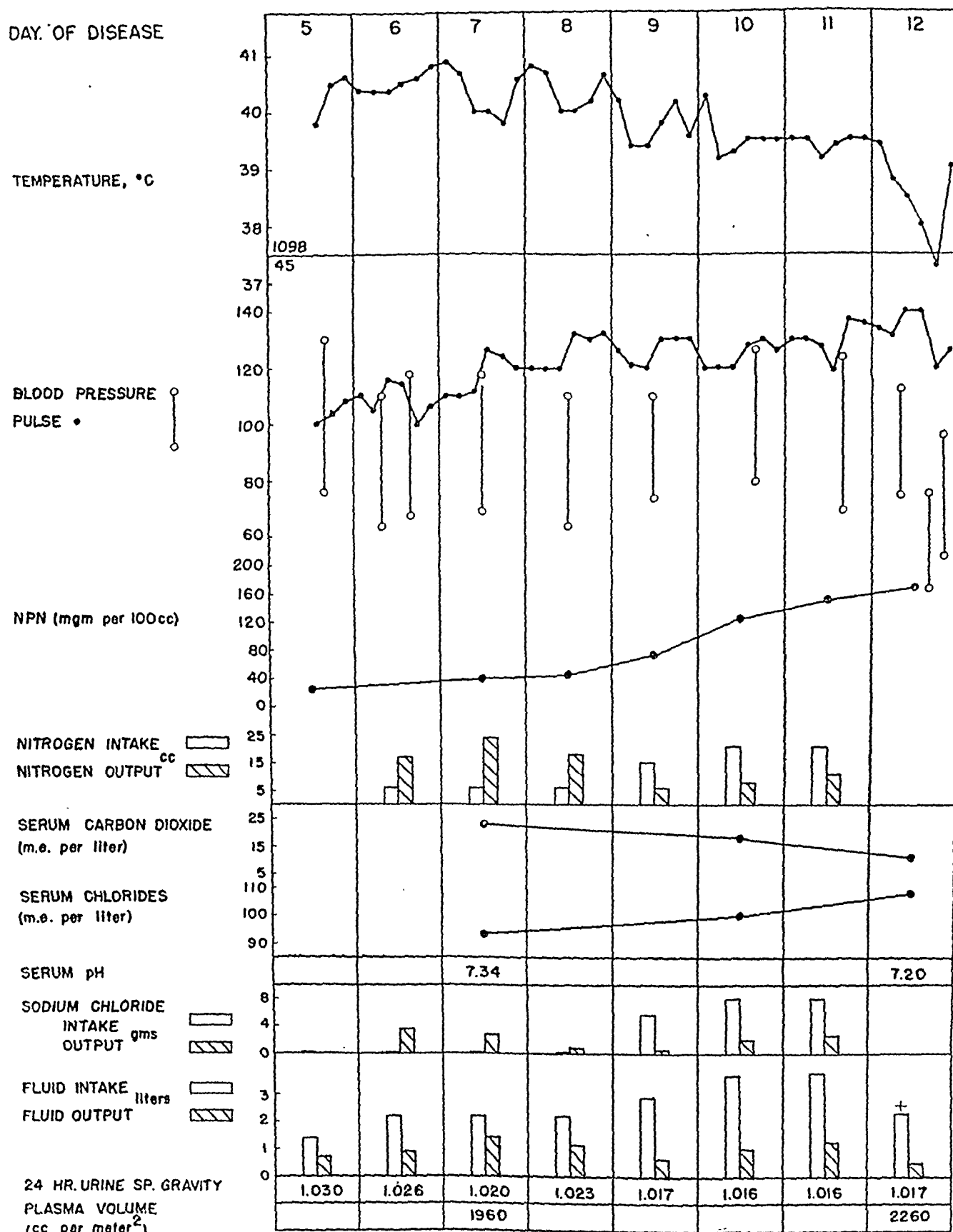
FIG. 1. THE CASE OF A 45-YEAR OLD MALE WHO ENTERED THE HOSPITAL ON THE 5TH DAY OF DISEASE

A rash was not present on admission but became profuse after the 6th day. In the absence of hypotension and with a normal plasma volume* and a daily urine output † of nearly one liter, he developed renal insufficiency.

TABLE II

Data on the chlorides and carbon dioxide content of the serum in patients receiving added salt in their diets

Case no.	Body weight	Average daily NaCl intake	Average daily NaCl output	Day of disease	Serum Cl	Serum CO2	Day of disease	Serum Cl	Serum CO2	Day of disease	Serum Cl	Serum CO2	Day of disease	Serum Cl	Serum CO2	Days of fever	Severity of case
					Before NaCl			After NaCl			After NaCl			After NaCl			
	kgm.	grams	grams		m. eq. per l.			m. eq. per l.			m. eq. per l.			m. eq. per l.			
446	58.3	11.5	11.0	9	88	24	13	103	22	15	108		20	126	24	20	E
3284	55.7	10.5	10.7	8	102	24	13	112	23							17	C
2501	54.5	9.5	4.0	3	96	25	13	107	23							31	E
3009	40.0	9.5	6.6	7	95	23	15	109	23							16	C
1174	54.5	9.5	8.2	9	97	24	15	104	25							10	B
1332	46.3	9.5	.6	6	85	20	9	103	20							11	F
447	51.3	8.5	6.5	9	87	26	11	96	25	16	97	26				16	F
666	46.0	8.5	1.6	8	96	21	12	114								21	C
1105	41.5	8.5	11.7	8	93	23	12	97	23	17	91	23				18	D
1014	—	9.5	6.5	10	88	31	16	98	23	19	101	26				18	D
1499	61.8	5.0	1.4	7	93	23	14	113	16							17	D
4246	48.7	6.5	7.4	8	98	23	10	98	25							12	F
1609	61.7	7.0	6.2				12	100	25							15	C
876	58.5	4.0	5.1	8	91	22	12	98	22	15	96	25				14	C
1178	56.0	6.5	.8	10	87	22	14	99	26							16	F
1845	54.0	6.5	1.3	9	96	23	13	102	25							14	C

11.6 m.eq. per liter, and the chlorides 108 m.eq. per liter. As this patient presented such interesting electrolyte changes, a detailed chart of his hospital course is shown in Figure 1.

It was generally the rule that when the serum chlorides were low the urine chlorides were either absent or greatly decreased. Case No. 4441 (Table I) was the only exception. This patient, in spite of low serum chlorides, continued to waste salt in his urine. When his serum chlorides were 80 m.eq. per liter, he excreted 4 grams of salt in a 24 hour collection of urine.

Eighteen patients were given "salt free" diets, and more than one determination of serum chlorides and carbon dioxide content was performed on 11 of them. It was noted in 5 cases that the chlorides rose during the 2nd or 3rd weeks of the disease despite the "salt free" diet. In 2 cases they fell, and in 4 cases they remained essentially unchanged. This is shown in Table I.

The addition of salt to the diet of the patients caused the serum chlorides to rise higher and more abruptly in the preceding cases (Table II). In a number of instances, the addition of 7 to 10 grams of sodium chloride to the daily diet with a natural salt content of no more than 1.5 grams effected abnormally high serum chlorides. This happened in cases 446, 3284, 3009 and 666 in Table II. Three of these patients continued to excrete most of the ingested salt, and yet the serum chlorides rose to abnormally high levels. This rise in the serum chlorides could not be accounted for by the difference in the salt intake and output. By including 4 to 7 grams of sodium chloride in the daily diet the serum chlorides were maintained within the normal range except in one case, and a good state of hydration was achieved. Patient 1499 who developed high serum chlorides on a daily intake of 4 grams of salt had renal failure. A summary of the effect of added salt on the serum chlorides is presented in Table II.

In a great many cases the sum of the carbon dioxide content and the chlorides of the serum was low. For that reason the total base was deter-

This was manifested by azotemia, a reduction in the 24-hour urine specific gravity and acidosis. The plasma volume was determined on the 12th day, and immediately after this the patient was given 1,400 ml. of isotonic human albumin intravenously in association with studies on the renal plasma flow. Seven hundred ml. of 10 per cent dextrose were given later in the day, and because of the severe degree of acidosis and accompanying dyspnea, 500 ml. of 10 per cent dextrose containing 25 grams of sodium bicarbonate were administered intravenously, but with no apparent effect. The patient died on the 12th day of disease.

* The plasma volume determination in this patient was performed by Comdr. R. A. Phillips, MC, USNR, using the blue dye (T–1824) method.

† The fluid output in all of these charts means urine output.

mined on several of these specimens. Serum proteins including albumin and globulin were determined in all cases. The results of these balance studies on 15 patients are shown in Table III.

The total base was normal in all cases except 5951, but this patient was receiving sodium bicarbonate. Serum calcium and phosphorus determinations were made in 10 cases. The values obtained were normal in all instances except one in which the calcium was slightly low. When the sum of the equivalents of carbon dioxide, chlorides, albuminates and globulinates was subtracted from the total base, an abnormally high value frequently was obtained for undetermined acids.

In view of the absence of severe azotemia in these cases, with the exception of case 4854, retention of the normal inorganic anions, $= HPO_4$ and $= SO_4$, is unlikely and it would appear that the increase in undetermined acids must have been largely organic. Studies to determine the exact nature of this undetermined acid were not made, however.

Comment. The low serum chlorides in the early stage of typhus require an explanation. It is likely that the diets of these patients before hospitalization were poor in salt. This change was not conspicuous, however, at corresponding stages in diseases other than typhus which were admitted to the Commission ward, *e.g.,* relapsing fever and typhoid. Perspiration was not a factor, as it rarely was observed in these typhus patients. This phenomenon was not peculiar to the dry climate

TABLE III

Acid-base balance studies of the serum in 15 typhus patients

Case no.	Age	Day of disease	Severity of case	CO₂ content (1)	Cl (2)	Alb./ Glob.	Albuminate (3)	Globulinate (4)	Total base	NPN	pH	Undetermined acid (total base minus 1+2+3+4)
				m. eq. per l.	*m. eq. per l.*	*grams per 100 ml.*	*m. eq. per l.*	*m. eq. per l.*	*m. eq. per l.*	*mgm. per 100 ml.*		*m. eq.*
1332	35	7	F	19.8	84.7	2.17 / 3.33	5.95	6.23	143.4	40	7.35	26.7
1499	23	6	D	22.7	93.3	3.85 / 2.43	10.9	4.7	138.9	35	7.42	7.3
1499		13		16.5	112.8	4.01 / 2.92	11.3	5.5	156.3	60	7.32	10.2
1584	35	16	D	22.8	95.4	3.14 / 3.28	8.6	6.2	155.7	38	7.34	22.7
1845	38	9	C	22.6	96.2	3.02 / 3.28	8.1	4.6	152.6	37	7.42	21.1
2140*	23	8	F	24.7	94.8				150.9	44		
2817*	18	4	C	25.1	94.9	3.90 / 2.67	11.0	5.1	153.2	31		16.8
3009	18	7	C	22.9	94.8	3.14 / 3.35	8.8	6.5	148.6	27		15.6
3100*	34	6	C	27.3	87.8	3.65 / 2.37	10.4	4.6	148.3	39	7.44	17.5
3713*	42	8	C	27.4	85.0	3.42 / 2.01	10.1	3.9	152.0	45		23.7

TABLE III—*Continued*

Case no.	Age	Day of disease	Severity of case	CO_2 content (1)	Cl (2)	Alb./ Glob.	Albuminate (3)	Globulinate (4)	Total base	NPN	pH	Undetermined acid (total base minus 1+2+3+4)
				m. eq. per l.	m. eq. per l.	grams per 100 ml.	m. eq. per l.	m. eq. per l.	m. eq. per l.	mgm. per 100 ml.		m. eq.
4441	18	12	D	24.7	88.6	3.26 / 3.36	9.0	6.5	143.2	29	7.37	14.4
4749	16	9	C	27.8	84.3	3.12 / 2.52	9.2	5.0	146.0	49	7.50	19.7
4854	18	10	F	16.4	82.3	3.46 / 2.44	9.6	4.7	140.5	124	7.37	27.5
4854		15		27.6	85.6	2.32 / 2.39	6.7	4.7	148.9	55	7.47	24.3
4934	35	8	F	24.9	81.9	3.37 / 2.66	9.7	5.3	144.9	27	7.47	23.1
5225	22	8	E	22.2	91.0	3.38 / 2.80	9.6	5.4	147.0	33	7.44	18.8
5225		16		20.8	96.0	3.39 / 3.41	9.5	6.6	148.7	31	7.40	15.8
5951	24	11	E	30.9	97.7	3.20 / 4.41	9.3	8.7	162.0	42	7.47	15.4

* Patients receiving para-aminobenzoic acid therapy; in these cases the m. eq. of para-aminobenzoic acid were determined and corrected for in the undetermined acids.

Note: The m. eq. of base bound to albumin and globulin was calculated by the following equation (10):

$$\text{Albuminate} = 0.125 \left(\frac{\text{grams alb.}}{\text{liter}} \right) (pH - 5.16)$$

$$\text{Globulinate} = 0.077 \left(\frac{\text{grams glob.}}{\text{liter}} \right) (pH - 4.89)$$

of Egypt. The failure of typhus patients to perspire was observed also by members of the Typhus Commission in moist climates. The salt was not lost in the urine, for the excretion of salt by that route was in relation to the concentration of the chlorides in the serum. When the serum chlorides dropped below normal, the urinary chlorides diminished markedly. The fact that the serum chlorides returned to normal spontaneously in spite of a poor salt intake is added evidence that the salt was not lost by the body, and suggests that the lowered chloride concentration was due to an expansion of the volume of extracellular fluid.

During the early stage of typhus the patients frequently have non-pitting edema. In the late stage of typhus, shortly before defervescence, there is often a fairly marked diuresis. These observations suggest that early in the disease there is an increase in extracellular fluid which would lower the serum chlorides, while later in the disease there is a loss of extracellular fluid and a diuresis resulting in a rise in serum chlorides.

The change in extracellular fluid volume was calculated in all cases where the data permitted. By assuming that the initial extracellular fluid volume was equivalent to 20 per cent of the body weight, change was calculated from the data on the salt balance and the change in the concentration of the serum chlorides. In the majority of these cases there was a loss in extracellular fluid during

SERUM PROTEINS IN LOUSE BORNE TYPHUS
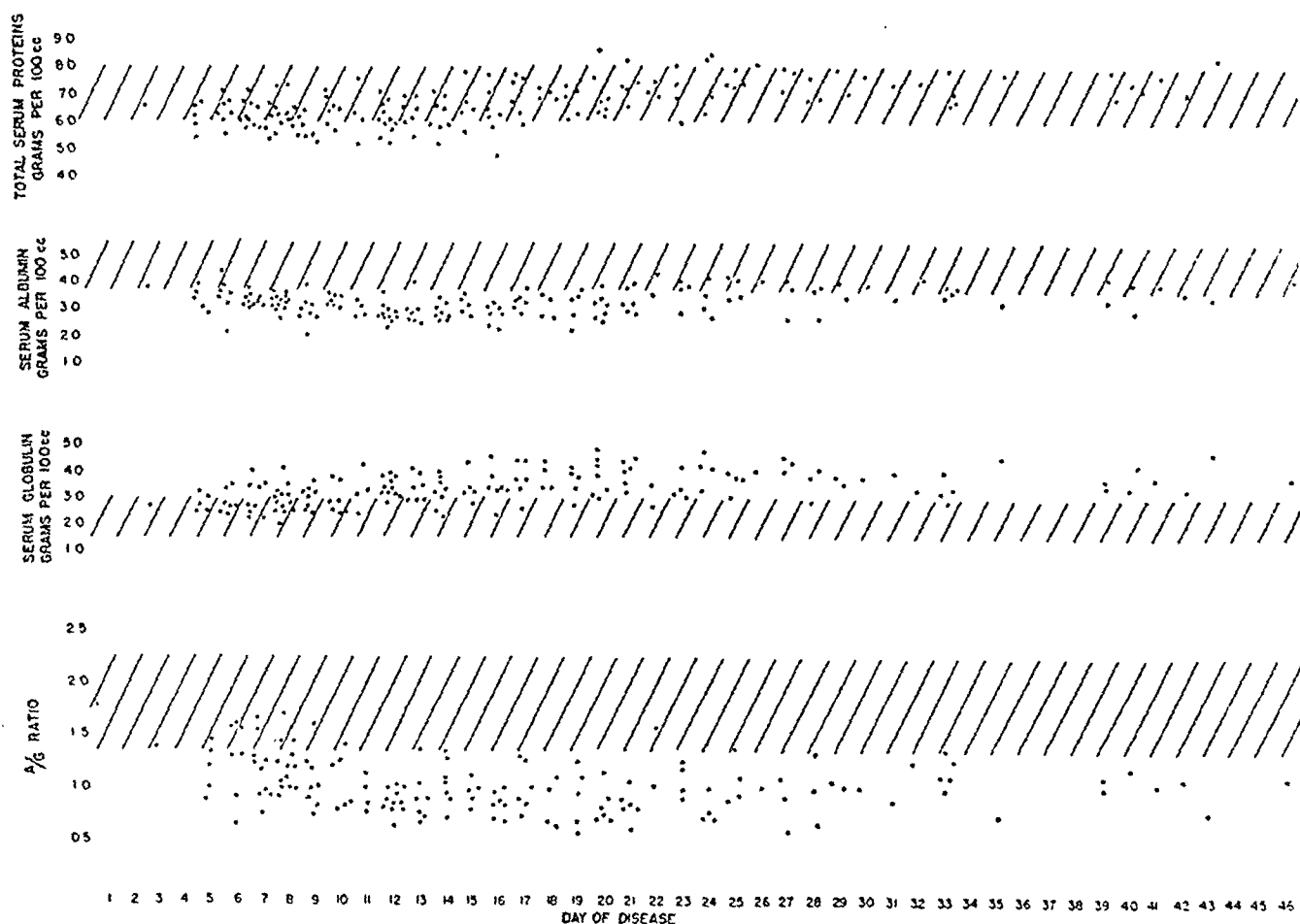
FIG. 2. THE LINED AREAS REPRESENT THE NORMAL RANGE OF VALUES

the period when the serum chlorides rose. Although the change in the calculated volume of the extracellular fluid had a definite bearing on the change of body weight in these patients, the relationship was not close quantitatively.

The raising of the serum chlorides to abnormally high levels by the daily ingestion of 7 to 11 grams of salt occurred in typhus patients with azotemia and renal insufficiency, as well as in typhus patients with apparently normal renal function. This differs from what usually occurs either in the normal individual or in the nephritic. The body weight of these typhus patients frequently could be increased as much as 5 pounds over a period of days by the administration of 7 to 11 grams of salt each day. In one case a slight amount of pitting edema was noted, but in the other cases the salt intake was reduced before this could take place. In view of the effect of large amounts of salt on the serum chlorides, one must be careful not to overload the patient with salt. It would

seem unwise to administer to typhus patients of this size more than 6 grams of salt a day without following the serum chloride concentration. A good example of the effect of salt on the patient's body weight is illustrated in Figure 8.

The increase in undetermined acid anions was as large as one encounters in severe metabolic acidosis. However, it was not reflected in the carbon dioxide content or in the pH of the serum, as both of these usually were within normal limits. Since the total base and the bicarbonate were within normal limits, the invading acid had apparently replaced chloride and albumin anions. The difference between this electrolyte pattern and the one in diabetic acidosis is shown by the diagrams in Figure 3; in typhus, serum reduction of $(BHCO_3^-)$ by the abnormal anion (X^-) is prevented by recession of (Cl^-). Therefore, the administration of ammonium chloride to typhus patients obviously is contraindicated.
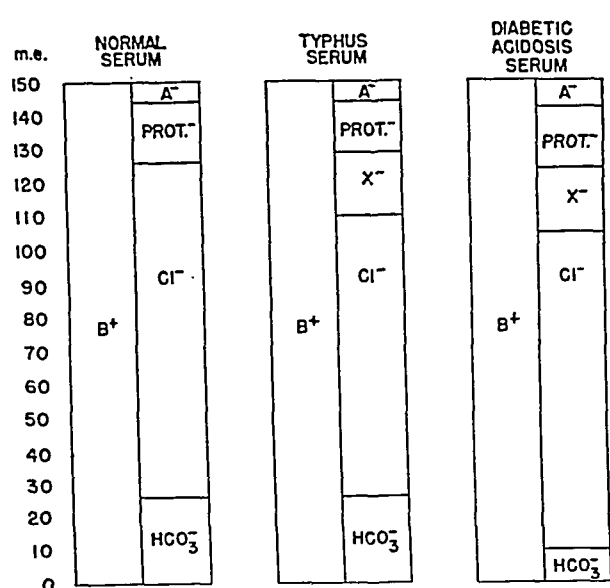
FIG. 3. THE ELECTROLYTE PATTERN IN THE SERUM OF TYPHUS PATIENTS AS COMPARED WITH THAT SEEN IN THE NORMAL INDIVIDUAL AND IN THE PATIENT WITH DIABETIC ACIDOSIS

"B +" represents total base. "A —" represents normal undetermined acid including phosphate and sulfate. "X —" represents pathological acids, and "prot —" represents the base bound to protein.

SERUM PROTEIN PARTITION

Specimens of serum obtained at different times during the disease and convalescence were analyzed for total protein, albumin and globulin. The results showed consistent abnormalities. The least change took place in the total proteins. Most of these were normal throughout the whole course of the disease, but a few were low during the first 2 weeks.

The albumin fraction was subnormal in almost all of the cases. This change was noted as early as the 5th day of disease, and usually persisted for 3 weeks. After that it rose gradually, but in some instances it was low even as late as the 5th and 6th weeks. When the albumin was below 2.5 grams per cent, the patients were always edematous.

The globulin fraction was abnormally elevated in most of the cases. This change occurred as early as the 5th day of disease. Throughout the course of the infection the globulins rose steadily. After the 3rd week when the temperature usually was normal they reached a plateau. At this time they were often above 4 grams per cent. This

represented a percentage increase from the initial to the final determination of over 40 per cent. If normal protein values had been obtained on these patients before the infection the percentage increase would have been still greater, as the value on the first determination usually was abnormally high.

The albumin-globulin ratio was usually low from the 5th day of disease on. It decreased further, and became reversed in the 2nd and 3rd weeks and it was still low even as late as the 7th week. The results of these studies are shown in Figure 2.

The percentage increase of over 40 per cent in serum globulins within a period of 3 weeks was a striking finding. High values for globulins often existed before the rash appeared or serological diagnostic tests became positive. As high values for serum globulins were seen rarely in conditions which could be confused with typhus, this abnormality helped to support the diagnosis of typhus early in the disease. No clear relation existed between the concentration of the globulins and the titres of the Weil-Felix or complement-fixation tests. It was noted that when the Proteus OX–19 agglutination titre was decreasing, the concentration of globulins frequently was increasing. Moreover, the change in serum globulins often occurred several days before serologic tests were positive. If the increase in globulins represents an increase in protective antibodies, the response occurs at an early stage in the clinical disease.

NITROGEN BALANCE

Results. Nitrogen balance studies were carried out on 21 patients. These studies covered periods during the acute stage of the disease and the convalescence. An attempt was made to study other patients along these lines, but due to the severity of the disease or to their failure to cooperate, insufficient data were obtained to warrant presenting. The gravely ill typhus patients, as a rule, were unable to tolerate the full quantity of the high protein, high caloric diet.

Of the 21 patients reported, 8 received high protein, high caloric diets, 10 received low protein, low caloric diets, and 3 received a combination of these diets. A summary of the results is shown in Table IV. Individual charts of selected cases are shown in Figures 4 to 8.
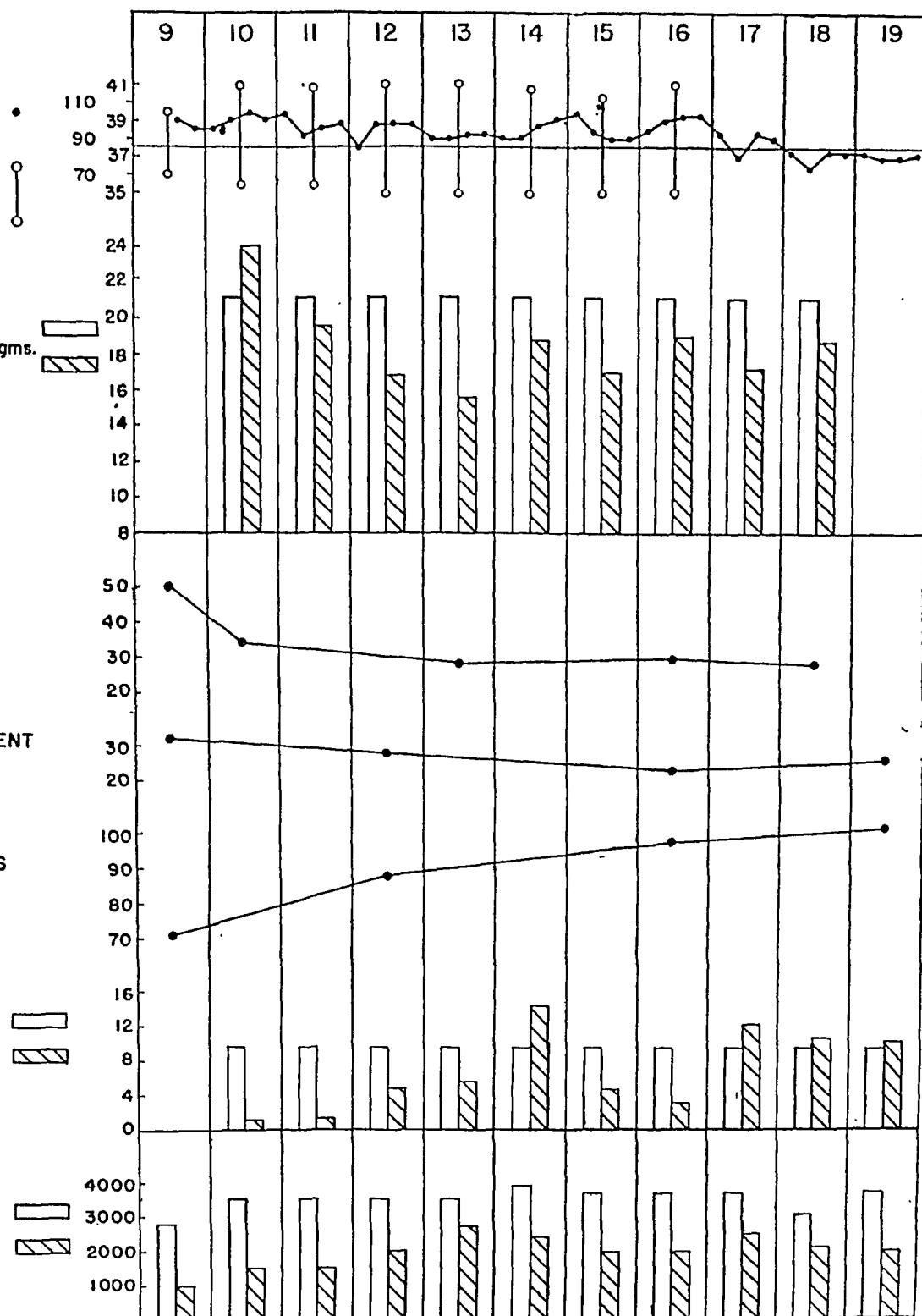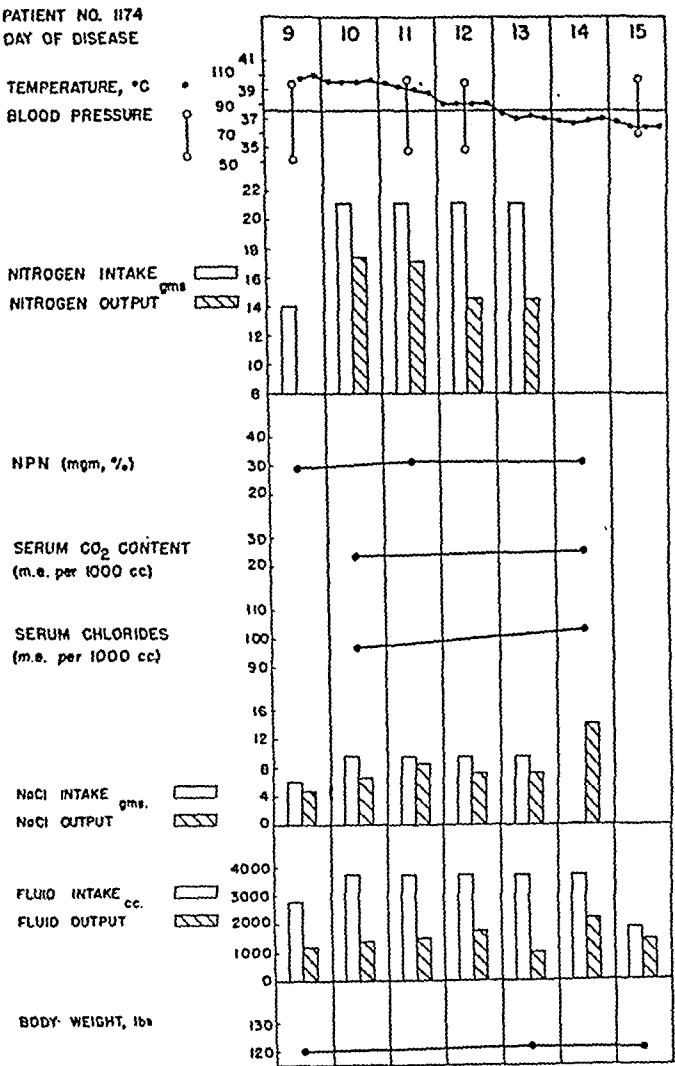
FIG. 4. PATIENT 1014, A 35-YEAR OLD MAN

This patient was admitted to the Commission ward on the 9th day of disease. He was given a daily diet containing 21.2 grams of nitrogen and 3,400 calories. He was acutely ill and febrile until the 18th day, but during this time he was kept in positive nitrogen balance except for one day, and his total nitrogen balance for this whole period was plus 22.4 grams. In convalescence he appeared well nourished.

TABLE IV

Data on the nitrogen balance during the febrile period in 21 typhus patients

Case no.	Age	Height	Adm. weight	Surface area	Day of disease diet started	Days on balance	Average daily nitrogen intake	Average daily nitrogen output	Total nitrogen balance	Average daily salt intake	Average daily salt output	Average daily urine volume	Highest blood NPN	Total change in weight	Days of fever	Severity of case
		cm.	*kgm.*	*sq. m.*			*grams*	*grams*	*grams*	*grams*	*grams*	*ml.*	*mgm. per 100 ml.*	*kgm.*		
4441	18	166	51.0	1.56	7	14	21.2	24.5	−46.2	3.0	7.5	2300	41	−1.8	25	D
3009	18	162	40.0	1.38	8	9	18.9	16.2	+24.3	7.7	6.6	1700	36	0.0	16	C
3284	25	163	55.7	1.59	7	10	17.9	22.0	−41.0	7.0	10.7	2000	50	−0.9	17	C
1105	32	164	41.5	1.41	8	8	20.3	16.8	+28.0	9.5	11.7	1500	31	0.0	17	C
1609	38	178	61.7	1.76	8	7	18.4	19.0	−4.2	9.5	6.2	2200	27	+0.3	15	C
1174	28	166	54.5	1.60	9	4	21.2	15.9	+21.2	8.6	8.2	1500	33	+0.4	12	B
696	20	155	51.5	1.47	10	5	17.4	17.0	+2.0	9.5	9.6	1750	35	−4.5	17	C
1014	35	171			10	8	21.2	18.4	+22.4	9.5	6.5	1900	33		17	D
1499	23	173	61.8	1.73	6	9.	12.8	31.9	−171.9	5.0	1.4	1800	96	−8.9	17	D
5225	22	164	56.9	1.60	7	10	14.0	23.0	−90.0	2.5	3.8	2300	41	−5.7	17	E
876	20	170	58.5	1.68	6	9	11.3	24.1	−115.2	3.3	5.1	1900	39	−5.7	14	C
5383	22	168	65.0	1.73	7	6	6.2	23.6	−104.4	6.2		2600	32	−2.5	13	C
777	12	140	35.0	1.16	8	9	4.1	12.0	−71.1	S. F.†	0.6	1500	39	−2.3	14	C
967	35	162	51.5	1.53	8	6	6.1	21.6	−93.0	S. F.	3.3	1700	63	−5.9	14	C
1020	36	168	59.8	1.68	9	9	6.1	23.8	−159.3	S. F.	1.8	1630	74	−4.1	18	C
746	17	163	42.0	1.42	10	5	5.9	16.2	−51.5	S. F.	1.2	1700	43	−4.1	17	C
1584	35	157	47.0	1.44	10	6	5.0	18.7	−82.2	S. F.	2.0	1800	59	−1.8	15	D
3713*	42	170	58.4	1.68	7	12	6.1	21.7	−187.2	6.0		2700	47	−4.1	32	C
4082*	23	175	64.0	1.78	7	10	6.1	22.6	−165.0	6.0		2650	38	−2.3	11	B
4601*	20	163	59.5	1.64	7	7	4.4	17.5	−91.7	5.0		1100	42	−1.3	13	B
4845*	27	161	51.3	1.52	8	11	5.9	18.1	−134.2	6.0		1800	40	−3.5	20	C

* These patients were receiving para-aminobenzoic acid therapy (8). In these cases the para-aminobenzoic acid was determined in the blood and urine and its nitrogen content was corrected for in the above values.

† Salt-free.

Note: Cases 4441 to 1014 on high protein diet; cases 1499 to 876 on a combination of high and low protein diets; cases 5383 to 4845 on low protein diet.

Note: The average daily salt intake listed here may differ from that listed in Table II because of the difference in time intervals over which the average was calculated.

Data are presented in Table IV showing the nitrogen intake and output, the non-protein nitrogen of the blood, and the weight change, as well as other pertinent observations. The daily fecal nitrogen is not recorded in the table as such, but it is included in the total nitrogen output. It varied from 0.5 to 4 grams daily. As can be seen in Table IV, the patients receiving low protein diets consistently had large nitrogen deficits and lost much weight. When the patients were receiving high protein diets, both the nitrogen deficit and the weight loss were markedly diminished. Five patients on the high protein diet had positive nitrogen balances during both the acute illness and the convalescence. Two patients actually gained weight during the acute illness, but some of this weight was due to increased hydration rather than deposited body fat or protein. The salt intake in these cases undoubtedly had an important influence on this weight gain. An example of this is shown in Figure 8.

The amount of nitrogen in the diet had no definite bearing on the total nitrogen excreted or on the non-protein nitrogen of the blood. Some of the patients receiving the low protein diets excreted more nitrogen than the patients on high protein diets. There was likewise no definite relationship between the occurrence of azotemia and the amount of protein destroyed. The urine output in these cases was between 1 and 3 liters daily. With normal renal function this amount should have been sufficient for the excretion of these excess nitrogenous products and the prevention of azotemia. We consider that patients 1499, 967, 1020 and 1584 had renal insufficiency.

In Table V the data on the nitrogen balance are presented on 8 patients who received the high protein, high caloric diet. This table is arranged to show the days when the patient was gaining or losing nitrogen. If the nitrogen intake were greater than the total nitrogen output on any day, the values were placed on the positive side of the
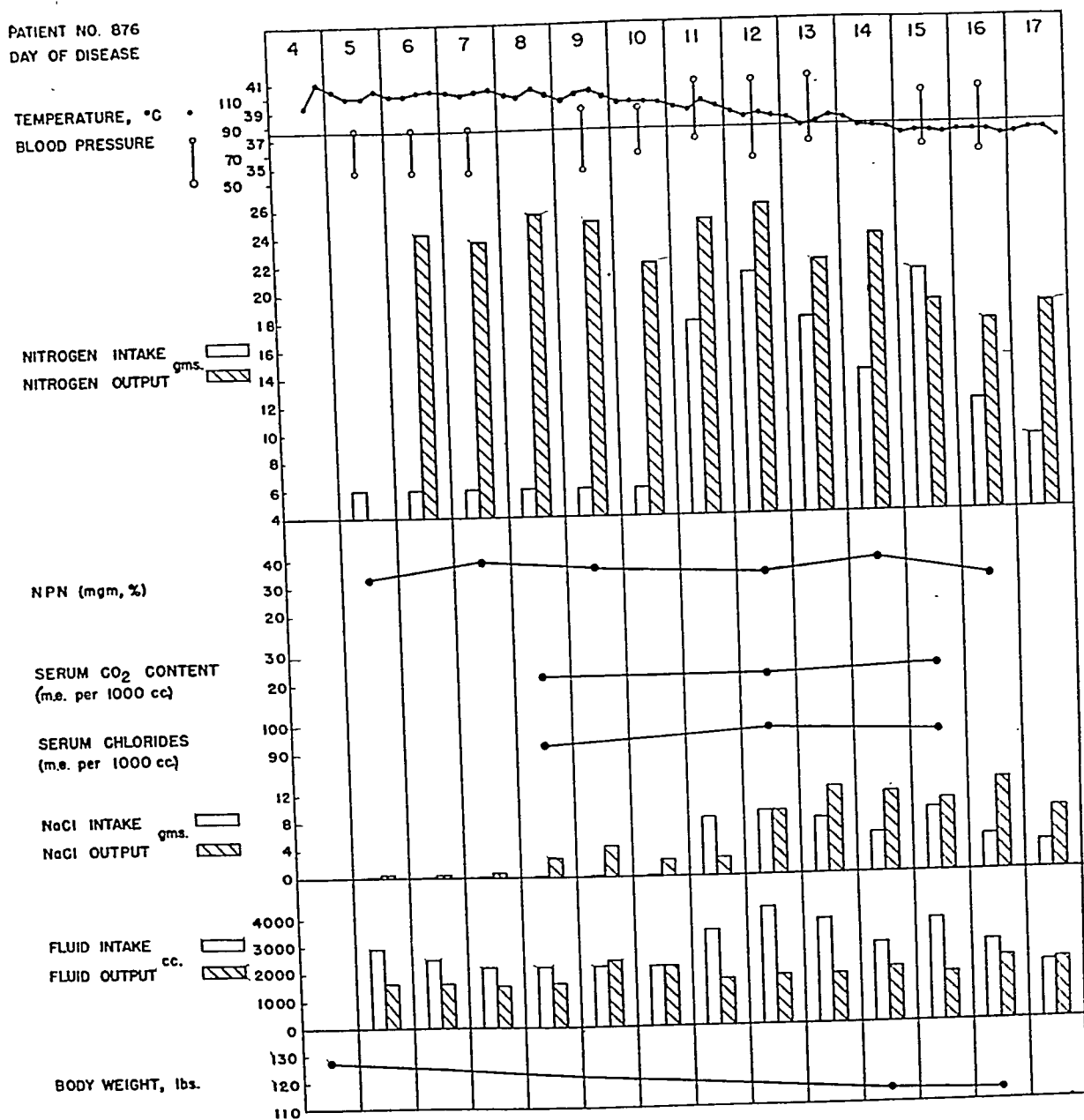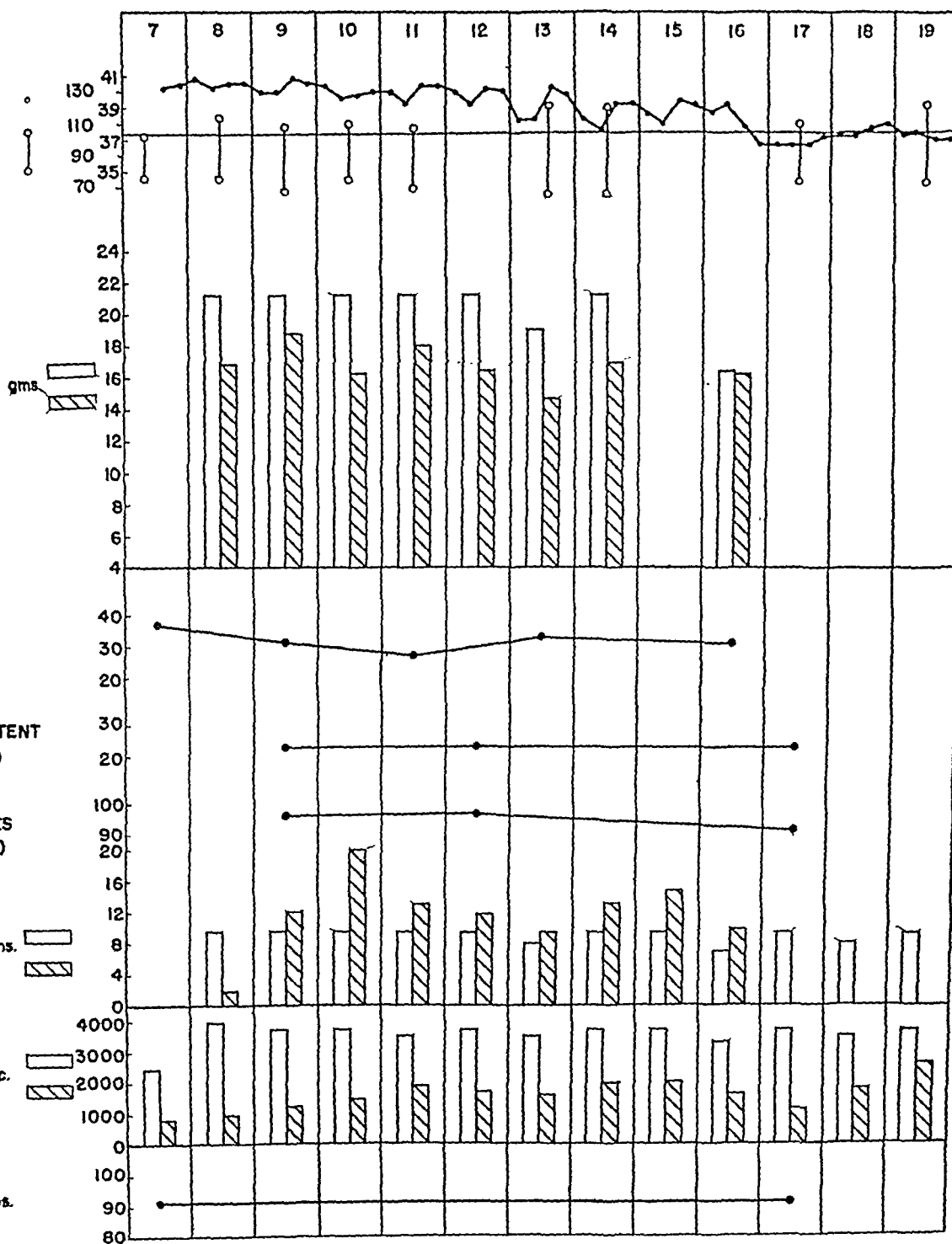
FIG. 5. PATIENT 1174, A 28-YEAR OLD MAN

This patient was admitted to the Commission ward on the 9th day of disease. He was given a daily diet containing 21.2 grams of nitrogen and 3,400 calories. He was acutely ill and febrile until the 13th day, but during this period he was kept in positive nitrogen balance and had a total nitrogen balance of plus 21.2 grams. The patient gained 1 pound during this period but this may have been due to the increased hydration influenced by the salt intake. The sodium chloride balance data are also included in this chart.

table; if the reverse were true, the values were placed on the negative side of the table. The total balance was obtained as follows: Total nitrogen balance = (nitrogen intake — nitrogen output × days on positive balance) — (nitrogen intake — nitrogen output × days on negative balance).

The type of diet had no noticeable effect on the changes observed in the serum proteins. The depression of the albumin and the elevation of the globulin occurred as frequently with high protein diets as with low protein diets.

The diet had no apparent effect on the course of the acute illness, but excluding the para-aminobenzoic acid cases, the patients who had received the high protein, high caloric diets appeared in convalescence, in most instances, to be stronger and much better nourished than the others. It appeared that they would be able to return to their work sooner because of this improved state of nutrition.

Comment. Many attempts have been made to prevent or mitigate wastage of nitrogen in acute infectious diseases. The results of such studies showed that in the majority of cases this could not be achieved. Only in chronic cases where there had been previous wasting could positive nitrogen balances frequently be obtained (11).

It has been demonstrated here that a diet high in protein and calories decreased wastage of nitrogen and loss of body weight during the acute phase of typhus. In 5 patients positive nitrogen balances were obtained during a period when the body temperature was around 39° or 40° C. Undoubtedly, these patients had large nitrogen deficits in the first week of illness prior to their hospitalization, and it is possible that this may have had some influence on their ability to store nitrogen at a later period. On the other hand, it is a well known fact that the 2nd week of the clinical disease is the critical period in the course of louse-borne typhus. During this week, patients show the greatest number of symptoms and signs of toxicity and central nervous system involvement. The majority of balance studies reported covered the period between the 7th and 14th day of the disease. Therefore, the data were obtained during the period in which these patients were most severely ill, and at this time they probably were destroying more protein than before. Thus, it seems unlikely in these cases than the presumed nitrogen loss during the first week of disease was an important factor in producing the positive nitrogen balance during the 2nd week of disease. The main reason for obtaining positive nitrogen balances in these cases appears to be the very high protein diet.

The 5 patients on whom positive nitrogen balances were obtained were receiving in their diet between 17.4 and 21.2 grams of nitrogen a day, which is the equivalent of between 109 and 132 grams of protein. This would be a high protein

FIG. 6. PATIENT 876, A 20-YEAR OLD MAN

This patient was admitted to the Commission ward on the 4th day of disease. He was given a daily diet containing 6 grams of nitrogen and 1,300 calories. This diet was kept up for 6 days during which time he was severely ill and had a high fever. On this low protein diet he excreted from 22 to 25 grams of nitrogen a day in a urine volume which ranged from 1.5 to 2.5 liters. In spite of the large amount of protein destroyed, the non-protein nitrogen of the blood remained normal. On the 11th day of disease the nitrogen intake was increased to amounts ranging from 14 to 21 grams a day, but there was no appreciable change in the nitrogen output. This patient was continually in nitrogen deficit during the febrile period, and had a total nitrogen balance of minus 115.2 grams. He lost 13 pounds during this time.

FIG. 7. PATIENT 1105, A 32-YEAR OLD MAN

This patient was admitted to the Commission ward on the 7th day of disease. He was given a daily diet containing 21.2 grams of nitrogen and 3,400 calories. He was severely ill and remained febrile until the 17th day. During this period he was in positive nitrogen balance, and had a total nitrogen balance of plus 28 grams of nitrogen.

TABLE V

Data on the nitrogen balance during the febrile period in 8 typhus patients on high protein diets

Case no.	Age	Adm. weight	Day of disease diet started	Negative nitrogen balance				Positive nitrogen balance				Total nitrogen balance	Average sodium chloride intake*	Total weight change	Days of fever	Severity of case
				Average daily nitrogen intake	Average daily nitrogen output	No. of days	Average daily nitrogen loss	Average daily nitrogen intake	Average daily nitrogen output	No. of days	Average daily nitrogen gain					
		kgm.		grams	grams		grams	grams	grams		grams	grams	grams per day	kgm.		
4441	18	51.0	7	21.2	26.8	10	5.6	21.5	19.1	4	2.4	−46.4	3.0	−1.8	25	D
3009	18	40.0	8	18.9	20.2	1	1.3	18.9	15.7	8	3.2	+24.3	7.7	0.0	16	C
3284	25	55.7	7	17.7	23.1	8	5.4	18.9	17.7	2	1.2	−40.8	7.0	−0.9	17	C
1105	32	41.5	8					20.3	16.8	8	3.5	+28.0	9.5	0.0	17	C
1609	38	61.7	8	17.1	23.5	2	6.4	18.9	17.2	5	1.7	−4.3	9.5	+0.3	15	C
1174	28	54.5	9					21.2	15.9	4	5.3	+21.2	8.6	+0.4	12	B
1014	35		10	21.2	24.0	1	2.8	21.2	17.6	7	3.6	+22.4	9.5		17	D
696	20	51.5	10	16.5	17.0	4	0.5	21.2	17.1	1	4.1	+2.1	9.5	−4.5	17	C

* The average daily salt intake listed here may differ from that listed in Table II because of the difference in time intervals over which the average was calculated.

diet for the average American man, but when one considers the size of these Egyptian patients, it amounts to a tremendous protein intake. Patients 3009, 1105, 1174 and 696 weighed, on admission 40, 41.5, 54.5 and 51.5 kgm., respectively, and their protein intakes were 2.9, 3.1, 2.4 and 2.1 grams per kgm. of body weight per day. Patient 1014 was not weighed on admission, but his weight on discharge was 55.5 kgm. His protein intake was 2.4 grams per kgm. of body weight per day. If patient 3009 had weighed as much as the average American man, *i.e.,* 70 kgm., and had received the same protein intake per kgm. of body weight, he would have received the huge quantity of 203 grams of protein per day.

The diet used in this study was composed of such foods as were available, and often there was not much choice. To many of the patients the diet was bulky and monotonous, and occasionally it contributed to nausea and diarrhea. With more foods from which to choose, smaller and more appetizing portions could be made up which would still be high in proteins and calories. On such a diet many typhus patients should ingest sufficient food to mitigate the great loss of body weight which occurs in this disease.

The outstanding feature of these studies of nitrogen balance was the very large and remarkably regular extent of protein catabolism regardless of intake. The average for nitrogen output (Table IV) was 20 grams per day which defines the consumption of 125 grams of protein. This is 3 to 4

times the minimal protein intake requirement under normal circumstances. However, since protein consumption is not found to be increased by intake, it can be covered provided the patient can accept food in sufficient quantity. This was demonstrated by the positive nitrogen balance obtained in 5 of the patients studied.

SUPPORTIVE PROGRAM

From these studies as well as our other observations in typhus, certain supportive measures are suggested for the rational management of the diet, electrolytes and fluid balance in the typhus patient.

(*1*) A liquid diet high in protein and calories should be administered. With diligent nursing the average patient will ingest at least 90 grams of protein and 2,500 calories a day. If the patient is too ill to take the diet, Amigen [4] may be given intravenously.

(*2*) The routine administration of large amounts of sodium chloride without determining the serum chlorides should be avoided. By including in the diet or in the parenteral fluids 4 to 6 grams of sodium chloride a day, the serum chlorides should be kept within normal limits and a good state of hydration achieved. Usually, the fluid intake should be maintained between 3 and 4 liters daily.

(*3*) The urine output should be at least 1 liter a day, and preferably 1.5 liters. A marked drop in the urine output is an ominous sign, particu-

[4] Casein hydrolysate manufactured by Mead Johnson and Company.
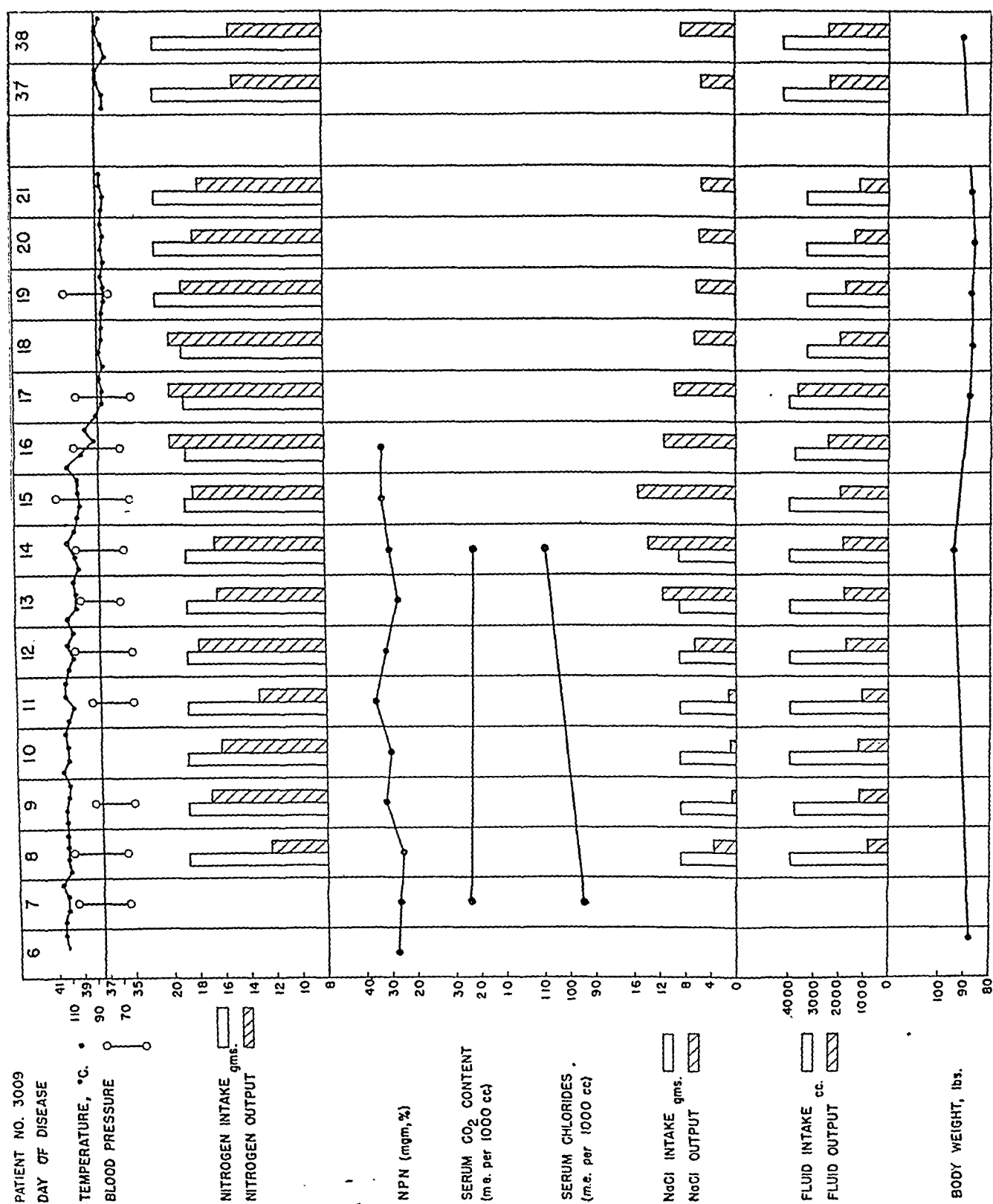
FIG. 8. PATIENT 3009, AN 18-YEAR OLD MAN

larly if it is associated with a fall in the arterial blood pressure (4). In such instances plasma or blood transfusions are required urgently.

(4) In cases of shock or impending shock, plasma or blood transfusions are indicated. The effect of plasma or blood transfusions on hypoalbuminemia is transient. In order to produce any significant change, very large amounts of plasma over a period of days would be required.

(5) Acid salts such as ammonium chloride, are obviously contraindicated because of the increase in undetermined acids in the blood.

CONCLUSIONS

1. A study of the electrolyte pattern of the serum revealed that early in the disease the chlorides were low in 62 per cent of the cases, but with few exceptions the carbon dioxide content was normal. The pH was normal in all of the patients except one who had renal failure and acidosis. The total base was normal in all of the cases studied except one. In all instances the undetermined acid anions were increased, and in the majority of cases they were as great as is encountered in severe metabolic acidosis.

2. The total serum proteins were normal in approximately 75 per cent of the patients, but the majority showed a depression of the albumin fraction and a very striking elevation of the globulin fraction. The increase in globulins represented a percentage increase of over 40 per cent in the average case.

3. There was no relationship between nitrogen output and intake or between protein destruction and azotemia. A high protein, high caloric diet decreased nitrogen wastage and loss of body weight during the acute phase of typhus. Positive nitrogen balances were obtained in 5 cases.

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EXPLANATION TO FIG. 8

This patient was admitted to the Commission ward on the 6th day of disease. He was given a daily diet containing 19 grams of nitrogen and 3,200 calories. He was acutely ill and febrile until the 17th day, but during this time he was kept in positive nitrogen balance except for 2 days, and his total nitrogen balance was plus 24.3 grams. The salt intake in this patient had a very noticeable effect on his body weight. On a salt intake of 9.5 grams a day the serum chlorides rose from 95 m.eq. per liter to 104 m.eq., and associated with this the body weight increased from 88 to 94 pounds. At this point all added salt was removed from the diet and in 3 days the body weight decreased to 88 pounds where it remained until late in convalescence.

CIRCULATING RED CELL VOLUME MEASURED SIMULTANEOUSLY BY THE RADIOACTIVE IRON AND DYE METHODS [1]

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The measurement of the circulating red cell volume is of considerable value in the study of the circulation in both the normal state and in experimentally induced abnormal circulatory states, and in disease. Modern modifications employing Evans Blue (T-1824) (1 to 3) of the original dye method of Keith *et al* (4) have clearly shown that plasma volume can be measured with a high degree of accuracy in normal man and animals. Values for normal plasma volume in man determined by several workers are in general agreement both as to absolute plasma volume and plasma volume per unit of body measurement (2, 5 to 7). The method has also proved reliable in the study of intravenous crystalloid (8) and colloid (9) therapy as well as in experimentally induced (10 to 12) and clinical shock (7, 12 to 14). A recent improvement in the method described by Noble (15), in which changes in dye concentration of blood samples are corrected for variations in water content as determined by serum protein measurements, should increase the applicability of the technique.

There is no general agreement among authors that the dye plasma technique measures either the total or circulating red cell volume. The opinion that cell volume can be calculated from the determined plasma volume and the hematocrit of blood samples drawn from large arteries and veins or the auricle, is based on the assumption that the hematocrit of blood flowing through the entire vascular bed is a constant at all times and under all conditions. Smith (16) found lower values

for cell volume when measured by carbon monoxide than by dye. Ebert and Stead (17) found cell volume determined by the dye method lower than the predicted volume after hemorrhage and during subsequent hemodilution. On the basis of subsequent experiments, these authors (18) concluded that the cell plasma ratio of blood contained in minute vessels is lower than that of venous blood.

Hopper (19) simultaneously measured cell volume by the dye and carbon monoxide methods in 13 normal humans and 17 normal dogs. The ratio of values by the former to the latter method averaged 1.00 in the humans and 1.08 in the dogs. The range of ratios in individual cases was from 0.91 to 1.16 in man, and from 0.72 to 1.14 in the dogs, and in each series the number of cases with ratios less than unity was about equal to those with ratios greater than unity. They found the ratios to be even more variable in abnormal subjects (20).

Root *et al* (21) made similar observations, and found little difference between the "central arterial and body hematocrit."

The red cell volume was first measured by means of radioactive iron in dogs by Hahn and coworkers (22). They found the cell volume measured by the injection of tagged cells consistently lower than the dye plasma cell volume, by as much as from 10 to 40 per cent, averaging 25 per cent.

In the course of studies on the preservation of human blood (23) we had occasion to determine the circulating red cell volume of normal young males by means of radioactive iron, and in many instances dye-plasma volumes were performed simultaneously. Similar studies were also made in a large series of normal (stray) dogs. It seemed worth while to present these data, obtained in a

large series of cases, since no similar studies have appeared in the literature.

METHODS AND MATERIALS

Plasma volume was determined by the method of Gibson and Evelyn (24). Blood samples were taken 10 minutes, and, in duplicate, 20 minutes after the injection of dye. Total blood volume and erythrocyte volume were calculated from the determined plasma volume and the venous hematocrit. Red cell volume was determined by the radioactive iron method (25). No correction was made for the injected donor cells (which are included in the cell volume when measured by radio-iron), since in most instances the quantity given was less than 2 per cent of the circulating cell volume. Donor cells were of Group O or A, and were cross-matched with recipient's serum in each case. All recipients were Rh positive. Blood donors were prepared either with the 5-year half life isotope (Fe^{55}) or with the 47-day half life isotope (Fe^{59}) but no donor had received both isotopes. Donor blood was drawn into acid citrate dextrose (ACD-G) (23) and refrigerated until used, and in no instance was the cell volume measured with donor blood that had stood (refrigerated) for more than 36 hours. Both Rh positive and Rh negative blood donors were used. The Fe^{59} donors had received their radioactive iron less than 100 days prior to the use of their cells in all instances.

Forty male medical students between 18 and 24 years of age volunteered for these studies. All had negative histories of blood dyscrasias, malaria, jaundice and recent acute infectious disease. No reactions following the injection of donor red cells occurred.

The recipient red cell unit activity due to the administration of tagged donor red cells (cpm. per ml. of red cells referred to the activity of a suitable standard of Fe^{55} or Fe^{59} measured at the same time) varied little. In most cases sampling was continued for 5 days after transfusion. The constancy of these levels is shown in Table I, which gives recipient unit activities at 20 minutes, 1 and 4 hours after infusion, and at 24-hour intervals during the following 5 days. These data were obtained in 5 consecutive experiments. The extreme ranges of deviation were 9.5

per cent above and 14.1 per cent below the average value. Of the 40 observations in Table I, 32, or 80 per cent, were within ± 5 per cent of the averages, and only 5, or 12.5 per cent, were more than ± 10 per cent of the averages. Hence the variations observed are for the most part within the probable error of the technique.

The plasma volume (Vpd), venous hematocrit, whole blood (Vwpd) and red cell volume (Vrpd) calculated from the plasma volume and hematocrit, the red cell volume determined by radio-iron (Vrr), and the sum of the plasma volume and radio-iron cell volume (Vwdr) are given in Table II. Also given is the ratio of the red cell volume as determined by radio-iron and dye-hematocrit (Vrr/Vrpd); and the body hematocrit, Vrr/Vwpd.

Red cell volume measurements were carried out simultaneously by both methods in 40 normal (stray) dogs. The dogs were of both sexes, and ranged in weight from 6.7 to 25.7 kgm. Fifteen animals were under nembutal anesthesia, and the rest were under light morphine narcosis. Results obtained are summarized in Table III.

RESULTS

In *every case* the value obtained for Vrr was less than that obtained for Vrpd. In the human series, the ratio Vrr/Vrpd showed extreme variations of from 0.70 to 0.95, the average ratio being 0.845. The standard mean deviation of the series was 0.72 per cent, the individual deviation 4.5 per cent. Seventy-five per cent of the cases had a ratio within ± 5 per cent of the average, and 90 per cent had a ratio within ± 10 per cent of the average.

In the series of dogs the ratio Vrr/Vrpd ranged from 0.62 to 0.98, averaging 0.825. The standard mean deviation of the series was 1.22, the individual deviation 7.59 per cent. Twenty-three per cent of the cases gave a ratio between .60 and .75; 39 per cent, between .75 and .85; and 28 per cent, between .85 and 1.0. The spread above and

TABLE I

Radioactivity* of recipient red cells following transfusion of cells tagged with Fe^{59}

Exp. no.	Days after transfusion								Average	Extreme deviation	
	0			1	2	3	4	5			
	20 min.	1 hr.	4 hr.							+ per cent	− per cent
70	.0207	.0191	.0214	.0205	.0203	.0201	.0206	.0206	.0204	4.9	6.4
71	.0207	.0213	.0191	.0228	.0215	.0226	.0241	.0241	.0220	9.5	13.2
72	.0234	.0243	.0246	.0233	.0219	.0242	.0223	.0222	.0231	5.3	10.9
73	.0229	.0218	.0207	.0233	.0241	.0229	.0217	.0232	.0228	5.4	14.1
74	.0403	.0377	.0425	.0433	.0433	.0408	.0405	.0408	.0413	4.8	8.7

* Expressed as Unit Activities = cpm per ml. of cells referred to cpm of a standard counted at the same time.

TABLE II

Plasma and circulating red cell volume determined simultaneously by the dye and radio-iron methods.
Normal males

Exp. no.	Date	Age	Hght.	Wght.	Surface area	Vpd	Venous hct.	Vwpd	Vrpd	Vrr	Vwdr	Vrr/ Vrpd	Body hct.
		yrs.	cm.	kgm.	m²*	ml.	per cent	ml.	ml.	ml.	ml.		
68	11-16-44	23	172	63.5	1.74	3110	43.4	5500	2390	1990	5100	0.83	39.1
69	11-17-44	20	180	69.0	1.86	3650	43.5	6280	2630	2100	5750	0.79	36.5
70	11-20-44	18	180	68.3	1.86	3850	40.8	6500	2450	1850	5700	0.76	32.4
72	11-24-44	21	175	72.5	1.86	3490	41.9	6010	2520	2040	5530	0.81	36.9
73	11-25-44	20	183	81.8	2.06	3930	43.5	6950	3020	2450	6380	0.81	38.4
75	11-30-44	20	172	66.0	1.77	3310	39.2	5440	2130	1810	5120	0.85	35.4
76	12-4-44	23	175	75.0	1.89	3850	42.7	6720	2870	2000	5850	0.70	34.2
77	12-5-44	22	188	78.4	2.03	3360	42.7	5860	2500	2380	5740	0.95	41.4
78	12-7-44	22	183	77.3	1.98	3330	42.1	5740	2410	2140	5470	0.89	39.1
79	12-8-44	22	173	65.4	1.77	3580	40.1	5980	2400	2040	5620	0.85	36.3
80	12-11-44	23	187	79.5	2.03	3320	40.2	5550	2230	2110	5430	0.95	38.9
81	12-12-44	22	174	63.5	1.76	3540	42.8	6190	2650	2150	5690	0.81	37.8
96	3-8-45	20	173	82.0	1.94	3520	38.5	5720	2200	1760	5280	0.80	33.3
97	3-13-45	22	173	63.3	1.76	2480	43.6	4400	1920	1610	4090	0.84	39.3
98	3-15-45	21	173	59.0	1.73	3370	43.0	5810	2440	1990	5360	0.82	37.2
99	3-20-45	20	179	65.5	1.82	2930	44.5	5280	2350	2010	4940	0.86	40.6
100	3-22-45	24	176	68.3	1.83	3070	43.4	5430	2360	2000	5070	0.85	39.6
101	3-7-45	24	186	84.2	2.08	3700	40.0	6180	2480	2260	5960	0.91	37.9
102	3-14-45	20	183	79.5	2.00	3430	42.8	6000	2570	2100	5530	0.82	38.0
103	3-12-45	23	174	70.2	1.83	3220	41.2	5750	2530	2050	5270	0.81	38.9
107	4-24-45	21	173	62.0	1.72	3180	44.0	5680	2500	2120	5300	0.85	40.0
108	4-25-45	22	173	89.0	2.06	3930	39.7	6520	2590	2200	6130	0.85	35.8
111	4-16-45	23	178	66.0	1.79	3390	42.0	5850	2460	2000	5390	0.82	40.8
113	5-7-45	23	193	80.0	2.08	5000	39.1	8200	3200	2760	7760	0.86	35.6
114	5-8-45	22	183	80.0	2.00	3850	43.3	6800	2950	2600	6450	0.88	40.3
148	8-13-45	22	183	80.0	2.00	3850	44.2	6900	3050	2480	6330	0.81	39.1
160	10-15-45	23	198	82.0	2.11	3740	42.7	6530	2790	2340	6080	0.84	38.5
162	10-22-45	20	189	84.3	2.10	4110	45.1	7480	3370	2910	7020	0.86	41.5
163	10-31-45	22	188	90.0	2.05	4180	43.0	7350	3170	2860	7040	0.90	40.7
164	10-29-45	21	174	66.0	1.82	2790	42.9	4890	2100	1870	4660	0.86	40.0
165	11-26-45	20	181	75.0	1.94	3230	45.0	5870	2640	2290	5520	0.87	41.4
167	12-3-45	23	194	84.2	2.12	4680	39.3	7710	3030	2540	7220	0.84	35.2
168	12-4-45	23	181	84.0	2.02	3400	40.0	5670	2270	1970	5370	0.87	36.7
169	12-11-45	24	194	89.0	2.18	3660	41.0	6220	2560	2160	5820	0.84	37.2
170	12-12-45	23	174	59.0	1.70	3200	45.0	5820	2620	2140	5340	0.82	40.1
172	2-27-46	20	175	66.0	1.78	2780	48.1	5360	2580	2180	4960	0.85	43.9
173	2-19-46	21	180	72.6	1.91	3980	43.2	7000	3020	2530	6510	0.84	38.9
175	2-25-46	21	173	84.3	1.97	3660	47.8	7000	3340	2920	6580	0.88	44.3
177	2-26-46	20	175	75.0	1.88	3800	40.0	6330	2530	2270	6070	0.89	37.4
178	2-21-46	20	181	76.5	1.96	3620	42.5	6300	2680	2350	5970	0.88	39.4
Average							42.44					0.845	38.44

* From Nomograms of Boothby and Sandiford.
Key: Vpd = Volume of plasma by dye method
          Vwpd = Volume of whole blood by dye method
          Vrpd = Volume of red cells by dye method
          Vrr = Volume of red cells by radio-iron method
          Vwdr = Total blood volume (Vpd + Vrr)

below the average was wider than in the human series.

This ratio, in individual cases, bore no relationship to venous hematocrit, or to absolute plasma volume or red cell volume (by radio-iron), as shown in Figure 1 for the normal males, and in Figure 2 for the dogs.

The body hematocrit (Vrr/Vwdr) in every case was lower than that of the venous hematocrit. The average of the body hematocrits was 38.3, that of the venous hematocrits being 42.5 in hu-

mans, and corresponding values were 41.6 and 46.8 in dogs. The ratio of body to venous hematocrit was 0.91 in the 2 series. Thus the body hematocrit is lower than the venous hematocrit by about 10 per cent. Since the ratio Vrr/Vrpd is an expression of the relationship of circulating cell volume to both plasma volume and hematocrit, it follows that the body hematocrit is independent of both the absolute plasma volume and hematocrit level.

Eight dogs were subjected to hemorrhages large

enough to produce considerable lowering of jugular hematocrits, but not to cause peripheral collapse over a period of a few hours to 3 days. Red cell volumes were measured by both methods simultaneously before bleeding and after hemodilution occurred. Four of the dogs were splenectomized. The data obtained are given in Table IV. The ratio Vrr/Vrpd was less than unity in all 20 determinations, ranging from 0.62 to 0.98 and averaging 0.82. Here again there was no correlation between the ratio Vrr/Vrpd and jugular hematocrit level.

Qualitatively similar observations were made in 2 patients in whom red cell volume was measured by both methods before and after transfusions of whole blood, and in 2 patients before and after phlebotomy (Table IV).

The relationship of normal blood volume to physical measurements is beyond the scope of this paper, and will be discussed in a subsequent communication.

DISCUSSION

The data presented consistently show that the circulating red cell volume, when determined by the radio-iron technique, is some 15 per cent less than when determined by the dye-plasma-hematocrit technique. A wider spread in individual val-

TABLE III

Plasma and circulating red cell volume determined simultaneously by the dye and radio-iron methods. Normal (stray) dogs

| Exp. no.	Date	Wght.	Vpd	Venous hct.	Vwpd	Vrpd	Vrr	Vwdr	Vrr/Vrpd	Body hct.	Body hct. Venous hct.
		kgm.	*ml.*	*per cent*	*ml.*	*ml.*	*ml.*	*ml.*		*per cent*	
131-4	2-19-42		1015	43.1	1780	765	570	1585	0.75	35.9	.83
131-6	2-26-42	14.2	670	50.8	1360	690	645	1360	0.93	47.3	.93
131-7	4-16-42	13.0	620	49.3	1220	600	560	1180	0.93	47.3	.96
131-39	3-10-42	13.6	630	50.4	1270	640	600	1230	0.94	48.7	.96
131-40	3-17-42	18.2	1105	45.7	2035	930	630	1735	0.68	36.6	.80
131-41	3-25-42	25.7	1415	47.1	2670	1255	980	2395	0.78	40.7	.87
135-7	7-22-42	20.0	1500	41.0	2540	1140	1000	2500	0.88	40.0	.97
135-8	7-29-42	16.5	1100	32.1	1620	520	450	1550	0.87	29.0	.90
135-9	7-29-42	15.5	1300	40.0	2170	870	790	2090	0.91	37.8	.95
135-11	8-5-42	15.3	765	47.7	1465	700	550	1315	0.79	41.7	.88
135-15	8-19-42	17.5	780	47.4	1480	700	625	1405	0.89	44.3	.94
135-16	8-26-42	21.4	1000	47.0	1890	890	665	1665	0.75	39.9	.85
135-89	2-11-43	21.0	910	51.3	1870	960	680	1590	0.71	42.8	.84
135-90	2-11-43	14.0	730	48.0	1400	670	645	1375	0.97	46.9	.98
135-96	2-18-43	16.5	975	48.2	1880	905	675	1650	0.75	40.8	.85
135-134	4-27-43	13.5	770	44.9	1400	630	525	1295	0.84	40.6	.91
135-136	4-29-43	9.3	390	52.6	820	570	385	775	0.68	49.8	.95
21-13	5-25-42	25.0	1125	61.3	2905	1780	1090	2215	0.62	49.1	.80
21-23	5-25-42	19.0	1235	38.9	2025	790	620	1855	0.79	33.4	.86
21-29	5-8-42	7.3	370	45.0	675	305	250	620	0.82	40.3	.90
21-41	6-18-42	16.1	800	48.8	1560	760	740	1540	0.98	48.0	.98
21-42	6-18-42	14.2	810	33.4	1215	405	320	1130	0.79	28.3	.85
21-45	6-18-42	18.0	1060	39.7	1760	700	465	1525	0.67	30.5	.77
21-102	7-13-42	9.0	475	45.4	395	870	315	790	0.80	39.9	.88
21-104	7-30-42	10.7	480	44.4	880	400	325	805	0.82	40.3	.90
21-105	7-30-42	9.3	400	49.9	800	400	300	700	0.75	42.8	.86
21-109	10-13-42	8.6	380	49.0	745	365	335	715	0.89	45.4	.93
21-113	12-8-42	18.4	945	46.7	1770	825	780	1725	0.95	45.2	.96
21-114	12-22-42	18.6	795	46.4	1480	685	620	1415	0.91	43.8	.95
21-117	1-6-43	20.5	1110	46.5	2070	960	830	1940	0.87	42.7	.91
21-118	3-8-43	17.5	990	47.7	2080	1090	890	1880	0.82	47.0	.98
21-119	3-24-43	16.8	910	39.6	1600	690	550	1460	0.80	37.7	.95
21-120	3-30-43	18.0	1130	41.4	1930	800	680	1810	0.85	37.5	.91
21-121	4-1-43	12.0	615	48.7	1050	435	360	975	0.83	37.0	.76
21-129	10-8-43	10.4	570	60.2	1430	860	670	1240	0.78	54.0	.90
21-130	10-19-43	10.9	640	47.6	1220	580	440	1080	0.76	40.7	.86
21-131	11-8-43	11.8	680	41.6	1165	585	440	1120	0.75	39.3	.95
21-132	12-8-43	16.4	915	50.7	1800	885	710	1625	0.80	43.7	.86
SA-2	11-3-43	6.7	515	41.7	870	370	330	845	0.89	39.0	.93
SA-3	11-17-43	9.0	285	52.0	595	310	290	575	0.94	50.3	.97
Average				46.8					0.823	41.6	0.91

NORMAL MALES
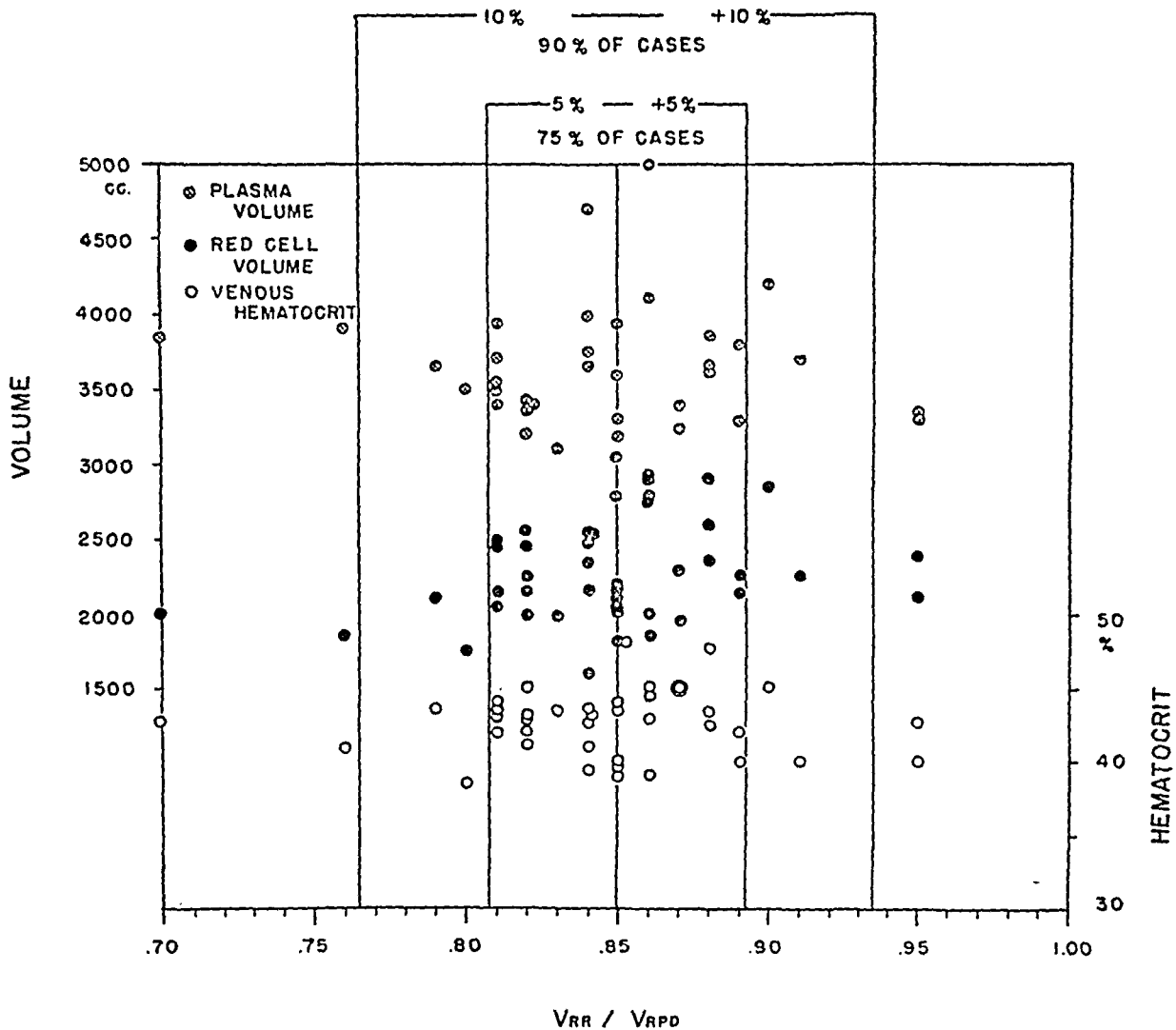
FIG. 1. THE RELATIONSHIP OF PLASMA VOLUME AND RED CELL VOLUME OF VENOUS HEMATOCRIT TO
THE RATIO OF RED CELL VOLUME, AS DETERMINED BY THE RADIO-IRON AND DYE METHODS

ues was encountered in the dogs than in the humans. These dogs were in varying states of nutrition and their past histories were unknown. Hahn (26) made similar observations in 8 normal dogs, in which the ratio of radio-iron to dye plasma red cell volume ranged from 0.64 to 0.91, averaging 0.79.

Hahn and Meneely (27) more recently carried out blood volume studies by the dye and radio-iron methods in 28 hospitalized patients,[2] with venous hematocrits ranging from 27.3 per cent to 50.6 per cent. In only 2 cases was the radio-iron cell volume higher than the dye-hematocrit cell volume, the average of the ratios thereof being

0.81. The average of the body hematocrits was 31.4, and the average of the venous hematocrits was 39.7, the ratio being 0.79, considerably lower than in our series. Both of these studies are, however, in keeping with our findings.

The values obtained by the radio-iron method are independent of variations in the hematocrit of blood samples drawn from large vessels, whereas the venous or arterial hematocrit is the basis of the calculation of red cell volume in the dye method. The consistent discrepancy in results obtained by the 2 methods requires that a decision as to which method most accurately measures the true circulating red cell volume be made.

The validity of the radio-iron technique rests on 2 assumptions, (1) that all of the tagged donor

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[2] The authors state "these patients were not normals, but people in various stages of disease or convalescence."

red cells remain intact throughout the period of significant observation, and (2) that all of the tagged cells become completely mixed with all of the recipient's cells within the vascular bed.

The donor cells used in these experiments were drawn in the best known blood preservative and, if not transfused immediately, were refrigerated until used. Under these circumstances little, if any, change in corpuscular measurements or in osmotic fragility occurs for at least 48 hours.

Hawkins and Whipple (28) estimated the normal life span of the canine red cell. Massive hemolysis was produced by phenylhydrazine, and this was followed by rapid regeneration of erythrocytes to a normal level over a 10 to 30 day period. Urobilinogen output fell off sharply during this period, and remained low until 100 to 120 days after cell regeneration had begun, when it rose abruptly, the rise being maintained for a period about equal to that during which regeneration had taken place. This rise was attributed to the

destruction of the cells regenerated after phenylhydrazine poisoning.

Shemin and Rittenberg (29) fed glycine tagged with N^{15} and found it resulted in the formation of heme with a high concentration of the isotope. They followed the N^{15} concentration of heme in human red cells for several months and concluded that the average life time of the erythrocyte is about 125 days.

Ashby (30) determined the life span of fresh red cells as being from 100 to 130 days by the agglutination method, in which Group O cells are injected into Group A recipients. These results have been repeatedly confirmed (31 to 35). There can be little doubt that freshly drawn compatible donor cells have their full life expectancy when administered to a recipient.

The second assumption is supported by the data presented in Table I. The recipients of these infusions of fresh Group O cells were leading normal daily lives: eating, exercising, and sleeping. The
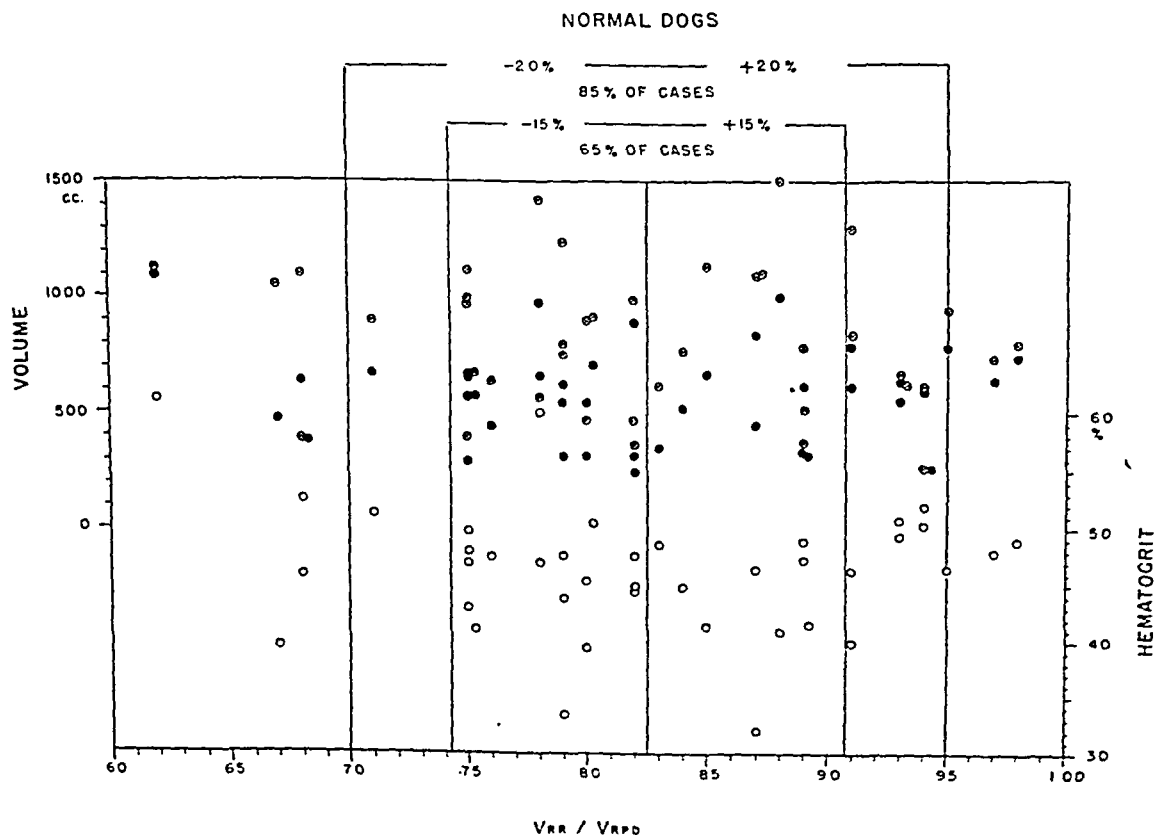
FIG. 2. THE RELATIONSHIP OF PLASMA VOLUME AND RED CELL VOLUME OF VENOUS HEMATOCRIT TO THE RATIO OF RED CELL VOLUME, AS DETERMINED BY THE RADIO-IRON AND DYE METHODS

occluded. Blood was then removed from a large vein until no more could be obtained. An Esmarch's bandage was then applied and additional blood from minute vessels was squeezed out. A comparison of the cellular content of the large and minute vessel blood was made on the basis of hemoglobin content. In 15 experiments the average hemoglobin content of the minute vessel blood was 13.2 grams per 100 ml., while that of the large vessels was 14.3 grams per 100 ml., the ratio being 0.9. Since some of the blood obtained by the final squeezing may have come from vessels larger than true capillaries, the hematocrit of very minute vessel blood may well be lower than the above ratio would indicate.

It is of interest to speculate to what extent the ratio of body to large vessel hematocrit remains constant at varying hematocrit levels. Hahn (36) found a linear relationship between jugular hematocrit and circulating red cell mass as determined by radioactive iron in individual dogs, within a range of from 11 to 57. This relationship may be expressed as the ratio of body to venous hematocrit. In both of our series there is a good correlation between body and venous hematocrit, within a range of from 38 to 48 for humans, and from 32.1 to 61.3 for dogs, as shown in Figure 3.

Thus it appears probable that the proportion of blood in large and minute vessels, and the hematocrits of the blood flowing through those compartments both remain fairly constant, within fairly narrow limits, under normal conditions. Direct evidence that this is the case will be presented in a further publication (37).

The significance of these findings is worthy of comment. The actual quantities of red cells involved in the intrinsic error of the dye plasma technique are not inconsiderable, ranging from 100 to 600 ml. in individual cases (Table II). This discrepancy is probably not too serious from a clinical diagnostic point of view, since the significant changes of cell volume in disease are frequently of a greater order. They do, however, become significant in clinical investigation, particularly in circulatory disturbances where the normal distribution of cells in large and minute vessels may be considerably disturbed.

CONCLUSIONS

1. Circulating red cell volume was determined by both the radioactive iron and dye-plasma methods

in 40 normal males and 40 normal (stray) dogs.

2. The ratio of the radio-iron to the dye-plasma red cell volume averaged 0.85 in humans and 0.82 in dogs.

3. The ratio of average body hematocrit to large vessel hematocrit averaged 0.91 in both series.

4. There is no relationship of either ratio to absolute plasma volume or large vessel hematocrit.

5. There is a linear relationship of body hematocrit to large vessel hematocrit.

6. The probability that the hematocrit of minute vessel blood is less than the body hematocrit is discussed.

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THE DISTRIBUTION OF RED CELLS AND PLASMA IN LARGE AND MINUTE VESSELS OF THE NORMAL DOG, DETERMINED BY RADIOACTIVE ISOTOPES OF IRON AND IODINE [1]

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Sufficient evidence, both indirect and direct, has now appeared in the literature, to leave no reasonable doubt that the hematocrit of all the circulating blood in the body is normally always lower than that of blood samples drawn from large arteries or veins, or from the right auricle.

The indirect evidence has been discussed in a previous communication (1). Direct evidence lies in the fact that the circulating red cell volume determined by radioactive iron is consistently less than when measured at the same time in the same subject by the dye-plasma-hematocrit method in dogs (2) and in humans (1). Equivocal evidence is the finding of either larger or smaller red cell volume by the carbon monoxide than by the dye technique (3).

The relationship of the average body to venous hematocrit has been shown to vary within fairly small limits ($\pm$ 5 per cent) (1), and the ratio of average body to venous hematocrit is a constant, within the limits between venous hematocrit levels of from 11 to 50 (4) in dogs, and from 38 to 48 in man (1). These facts imply a fairly constant distribution of blood within large and minute vessels in the normal state. It therefore seemed advisable to make quantitative measurements of the quantity of plasma and red cells flowing through the large and small vessels in relation to the total measurable blood volume. The development of techniques of tagging red cells with radioactive iron (5) and plasma with iodine (6) made a di-

rect attack on the problem possible. The studies on normal dogs reported herewith were undertaken as a control for subsequent work on the intravascular distribution of blood in experimental shock.

METHODS

All dogs were fasting (24 hours) and were maintained under light morphine narcosis (2 mgm. per kgm.) throughout the observation period. An initial plasma volume determination was made by the method of Gibson and Evelyn (7). At the same time, the dog received an infusion of freshly drawn compatible dog red cells tagged with Fe^{55}, for the determination of circulating red cell volume. The dog was then allowed to walk about the laboratory at will. Three to 5 hours later, plasma volume was again determined, and an infusion of freshly drawn compatible dog red cells tagged with Fe^{59} was given. At the same time an infusion of bovine albumin or fresh dog plasma iodinated with radioactive iodine (I^{131}) was given intravenously. The stability of the iodine linkage in such iodinated plasma protein, as well as the method of preparing and measuring its radioactivity in blood and tissues, is given in a previous publication by Fine and Seligman (6). Samples of blood were taken throughout a 1-hour period for measurement of plasma dye level and iodine radioactivity, measurement of the hematocrit and hemoglobin concentration of whole blood in the large vessels, and of the radioactivity level of both isotopes of iron in the red cells. The dog was then sacrificed by the intravenous injection of nembutal.

The animals were autopsied immediately after death, and the weight of the organs was recorded. Organs analyzed were spleen, liver, lung, kidneys, heart, bowel, muscle and brain. No bone marrow was obtained. The organs were not perfused, and no attempt was made to prevent blood from oozing out as the organs were cut up. Small pieces of tissue which avoided large vessels were gently wiped with gauze and weighed.

Representative samples of each organ were taken and finely divided. An aliquot of each organ was analyzed for hemoglobin (6), radioactive iodine (6), and both isotopes of radioactive iron.

The content of plasma and red cells in the small vessels of each organ was calculated, in ml. per gram of tissue (6). The unit quantity of plasma was taken as the same proportionate radio-iodine activity of the tissue sample as the radio-iodine value of plasma samples at time of death was to the extrapolated plasma activity at the time of injection. The assumption was made that iodo-protein loss from the circulation between the time of injection and death occurred in all tissues to the same extent. The unit quantity of *total* red cells was based upon the relative concentration of hemoglobin in whole blood and that obtained by extraction of hemoglobin from tissue samples, as described by Fine and Seligman (6). The unit quantity of cells tagged with Fe^{55} and Fe^{59} respectively was based upon the relative radio-iron activities of both isotopes of a red cell sample drawn just prior to death, and the tissue sample. In making radio-iodine measurements in tissue, corrections were made for radioactivity due to radio-iron from red cells, but these were not significantly great in most instances. In 4 experiments, total red cell content was calculated from extracted hemoglobin values, and in 3 experiments from Fe^{55} values. Rapidly circulating red cell content was based upon Fe^{59} measurements in all instances. Total plasma and red cell content was taken as the product of unit content and organ weight. Iodine measurements reflect only circulating plasma.

The net amount of whole blood removed in sampling was less than 5 per cent of the initial total blood volume, and no significant changes in mean arterial pressure were observed. No correction of tissue values for bleeding were made.

The protocol of a typical experiment is given below, and illustrated in Figure 1. Therein is shown the ex-pected change from initial values in plasma, cell and whole blood volume due to the administration of whole blood and radio-iodo plasma for cell and plasma volume determinations. The determined final volumes were within 4 per cent of the expected values.

Table I summarizes the findings in Experiment No. 25–131. The unit values (ml. per gram of tissue) for plasma (by radio-iodo-protein), for total red cells (by Fe^{55}) and for circulating red cells (by Fe^{59}), as well as for whole blood (the sum of plasma and circulating cells), are given for each of the 8 organs analyzed. The minute vessel content of plasma, red cells and whole blood is the product of the respective unit value and the weight of the organ. Also shown is the ratio of circulating to total red cells in the minute vessels for each organ, and also the hematocrit of whole blood for each organ. The minute vessel hematocrit was calculated from the circulating red cell content and the whole blood content.

The percentage of minute vessel content to total volume was 16.7 for plasma, 13.3 for circulating red cells, and 16.3 for whole blood respectively. The quantity of circulating red cells in minute vessels of all the organs was approximately equal to the quantity of total red cells. The weighted ratio of circulating to total red cells was 1.05; the arithmetic average of the ratios of the several organs was 1.02.

The arterial hematocrit at the time of final volume measurement was 42.1, the hematocrit of all the blood in circulation was 40, while that of the blood in all the minute vessels was 32.6.

A comparison of the values obtained in 4 experiments for the final plasma volume both by dye and by radio-iodo-protein is given in Table II.

Protocol. Normal dog 11.8 kgm.
Exp. No. 25-131

11/8/43

Time		Hct.	Given			Bled			M.A.P.	Vrr	Vpd	Vrwp
			Red cells	Plasma	Whole blood	Red cells	Plasma	Whole blood				
		per cent	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*mg. Hg.*	*ml.*	*ml.*	*ml.*
8:35 A. M.	Morphine sulphate 23.6 mg. i.m.								95			
10:30	Blood tagged with Fe^{55} i.v.	45.8	32	38	70				100			
10:46	3 cc. 0.1% Evans Blue i.v.											
11:25	Samples for volume I complete	41.6				27	38	65	440	675	1115	
11:28	Dog off table											
2:00 P.M.	Dog on table											
2:10	Pre-volume II samples					6	9	15				
2:12	Blood tagged with Fe^{59} i.v.	41.3	29	36	65				110			
2:15	Radio-iodo-albumin i.v.			14								
2:28	3 cc. 0.1% Evans Blue i.v.											
3:08	Samples for volume II complete	42.1				23	22	45	110	460	690	1150
	Net change due to transfusion and bleeding *			+13	+9	−4						
	Expected volumes									436	692	1128
3:09	20 cc. nembutal i.v.											
3:11	Animal expired											

* Computation does not include first transfusion or blood removed for samples after injection of radio-iodo-albumin.

In Table III are shown the unit values for total red cells of the organs studied as measured by hemoglobin in 5 experiments and by Fe^{55} in 2 experiments. The spread of values for individual organs obtained by hemoglobin measurement was greater than that obtained by radioactivity analysis. The average unit values for the individual organs based on radioactivity measurements were larger than that based on hemoglobin measurements in 4 organs, and smaller in 4 organs. The averages of the unit values for each organ, however, are in essential agreement. The largest errors in hemoglobin technique were with myoglobin-containing tissues (skeletal muscle).

Table IV summarizes the findings obtained by the experimental procedure described in 7 normal dogs. Some of the data included were presented in a previous communication (8).

The range and average of intravascular content of red cells in minute vessels includes all values found by both hemoglobin and radio-iron for both total and circulating red cells, since, in the entire series of experiment, the ratio of circulating to total cells was found to be unity. Therefore the average unit values for the individual organs are somewhat different than the average values shown Table III, in which only values for total cell values we compared.

The range of unit values for whole blood also repre sent extremes of red cell unit values and hence are n the arithmetic sums of average red cell and plasma un values.

The per cent of total red cell plasma and whole bloo in minute vessels was calculated from the circulatin plasma and red cell volume and the final gross plasm and red cell volumes.

The values given for the hematocrit of blood in th minute vessels of each organ are arithmetic averages fo the whole series of dogs. The arterial body and minut vessel hematocrits also are arithmetic.

The ratio of rapidly circulating to total red cells wa 1.01 ± 0.05.

An average of 16.5 per cent of total red cells 20.4 c total plasma and 17.2 per cent of whole blood was foun in the total minute vessels of the organs analyzed.

Table V shows the hematocrit of whole blood in arte ries, in large vessels, in the entire body, and in minut

EXPERIMENT N°. 25-131   NORMAL DOG 11.8 Kg.   November 8, 1943
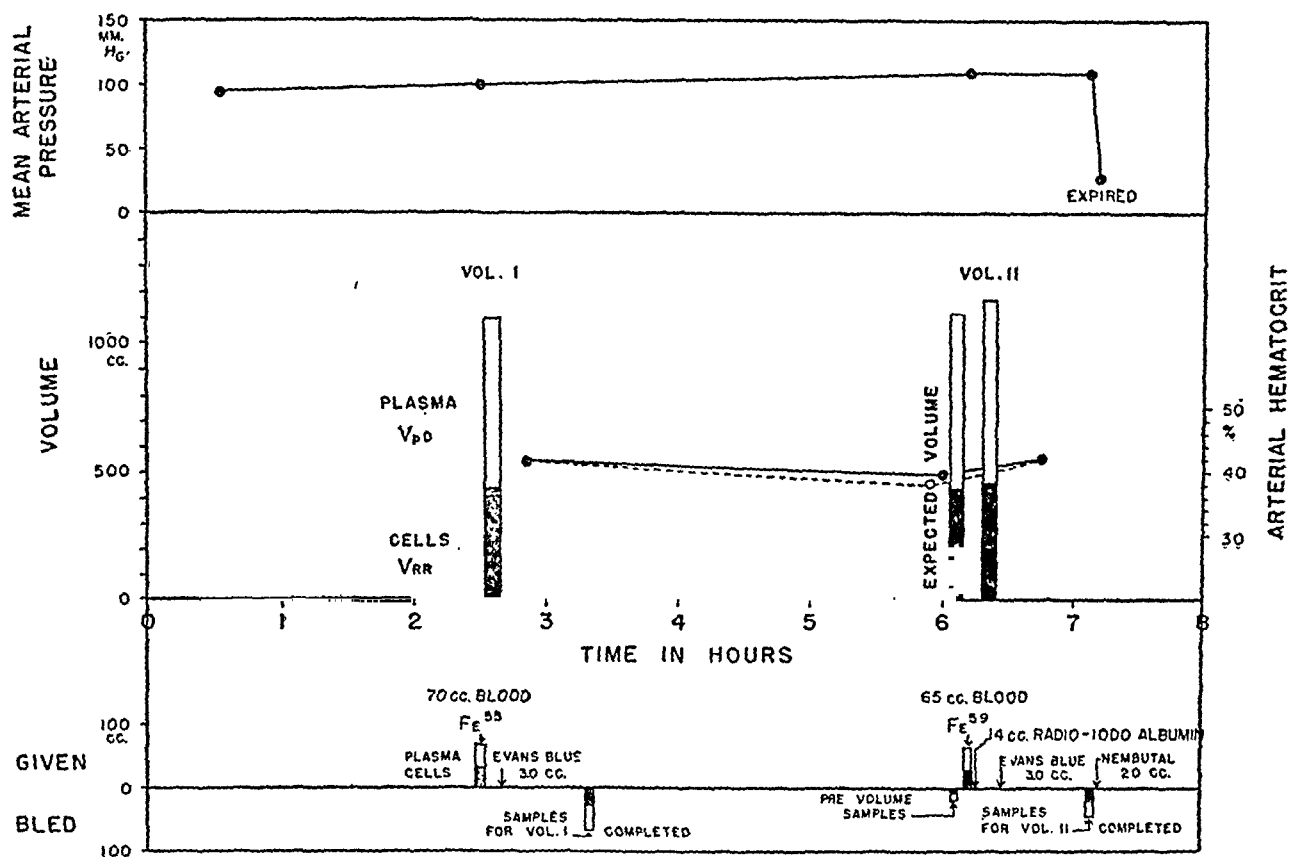
FIG. 1. TIME CHART

This shows injection of erythrocytes tagged with Fe^{55} and Fe^{59} for determination of red cell volume, Evans Blue for determination of plasma volume, and radio-iodo-albumin for measurement of minute vessel plasma content. The final cell and plasma volume agreed well with the expected final volumes.

TABLE I

The unit quantity and total content of plasma and red cells in the minute vessels of a normal dog

Experiment No. 25-131.   Normal Dog 11.8 Kg.   Morphine narcosis.
November 8, 1943

Organ	Weight	Plasma	Red cells		Organ minute vessel content				f2†	Minute vessel Hct.*
			Fe⁵⁵	Fe⁵⁹	Plasma	Red cells		Whole blood*		
						Total	Circulating			
	grams	ml. per gram	ml. per gram	ml. per gram	ml.	ml.	ml.	ml.		
Spleen	72	.068	.393	.442	4.9	28.20	31.80	36.70	1.12	87
Lungs	99	.201	.107	.100	20.0	10.60	9.90	29.90	0.93	33
Liver	252	.128	.020	.019	32.3	5.05	4.80	37.10	0.95	13
Kidneys	58	.074	.006	.007	4.3	0.35	0.40	4.70	1.16	9
Heart	83	.051	.015	.015	4.3	1.25	1.25	5.55	1.00	22
G. I. tract	350	.035	.006	.006	12.3	2.10	2.10	14.40	1.00	15
Muscle‡	3540	.0103	.003	.003	36.5	10.60	10.60	57.10	1.00	19
Brain	62	.0109	.003	.003	1.8	0.18	0.18	1.98	1.00	9
Totals	4516				115.4	58.33	61.03	187.43		
Averages									1.02§	32.6‖
Final volumes					690.0		460.0	1150.0		
Percentage of total volume in minute vessels					16.7		13.3	16.3		
Percentage of total volume in large vessels					83.3		86.7	83.7		
Body hematocrit (final volume)										40.0
Arterial hematocrit (final volume)										42.1

* Based on plasma and circulating cell content.
† Ratio of circulating to total red cells.
‡ Estimated as ⅓ of body weight.
§ Arithmetic average of individual f2 values.   Weighted average is 1.05.
‖ Based on total circulating red cells and plasma in minute vessels.

vessels. The averages of the ratios of these hematocrits to the arterial hematocrits were 0.92, 0.85 and 0.68 respectively.

DISCUSSION

Several aspects of the techniques used warrant consideration.

Plasma volumes were calculated from radio-iodo-protein data in 4 experiments. The values were within ± 10 per cent of the values obtained by the dye method (Table II), and this agreement constitutes a check on the reliability of the unit

TABLE II

Plasma volume measured by both the dye method and by radio-iodo-albumin

Exp. no.	Plasma volume		Difference	Error*
	By dye	By radio-iodo-albumin		
	ml.	ml.	ml.	per cent
111	1390	1335	−55	−4
113	990	1180	+90	+9
114	800	840	+20	+5
127	690	670	−20	−3

* Percentage by which plasma volume by radio-iodo-protein differed from value by dye method.

values of plasma in the minute vessels of the organs analyzed. The tagged red cells transfused were compatible with the serum of the recipient dog. These red cells were freshly drawn in heparin, and there was no evidence of any breakdown of those cells in the subject animal. Consequently, all the measured radioactivity of iron of the samples derived only from intact erythrocytes, and none from tissue iron.

The radioactivity measurements obtained were sufficiently higher than instrumental background to be significant in all samples of both red cells and organs. The probable error of the lowest measurements was no greater than that of the highest measurements.

The manner in which the organs were cut up was such that only blood in very small vessels was included in the samples analyzed. Brain, heart, kidneys and spleen were completely divided into pieces measuring not over a few mm. in the longest dimension. Portions of each lobe of the liver, of each lobe of the lungs, of the cardiac and pyloric portions of the stomach, sections of duodenum, jejunum and colon, and sections of neck,

TABLE III

*Total red cell content of minute vessels as measured by hemoglobin and by radioactive iron analyses of tissue samples ** *

Exp. no.	100	109	111	113	114	127	131	Averages	
	By hemoglobin					By radio-iron		By hb	By radio-iron
Spleen		.370	.283	.340		.338	.363	.331	.350
Liver	.100	.030	.038	.035	.018	.057	.020	.038	.038
Lungs	.160	.053	.057	.074	.050	.081	.107	.080	.094
Kidney	.010	.050	.046	.046	.048	.032	.040	.040	.036
Heart	.015	.006	.013	.006	.012	.013	.015	.011	.014
G. I. tract	.027	.040	.004	.013	.005	.027	.006	.020	.016
Muscle	.006	.006	.005	.007	.004	.004	.004	.006	.004
Brain	.002					.003	.003	.002	.003

* All values are for ml. of red cells per gram of tissue.

back, abdominal and both leg muscles were cut up in the same way. The organs were not perfused, nor was their blood supply tied off prior to removal from the body. All of the blood that would was allowed to drain out during sectioning, but the cut pieces were not squeezed; the surfaces were gently wiped. Visible vessels were excluded from samples. The pieces of organ in the aliquot processed were selected to be representative of all parts of the organ. Thus it was felt that the blood examined came from vessels not larger than arterioles or venules. Bone, bone marrow, fat, and the mesentery were not examined. Since the skin of the dog was found to be virtually bloodless, it was not routinely assayed.

The total muscle weight was estimated as ⅓ of the total body weight, since that was the proportion found in many dogs in another study (9) in which practically all of the skeletal muscle was removed from the bones. It was felt this estimate was sufficiently accurate for our purpose, so that this laborious and time-consuming process could be dispensed with.

It was assumed that hemoglobin extracted from tissue samples was an accurate measure of the total red cells in the blood vessels, except in the case of muscle samples which contain myoglobin and therefore required separate extraction of myoglobin with saline before extraction of hemoglobin with water (6).

In 5 experiments, the total red cell unit value was based on the hemoglobin content; and in 2, on Fe[55] radioactivities. The red cells were leached out of very finely divided, but not crushed, pieces of tissue with water in the cold for 24 hours.

Hemoglobin was measured with the Klett photoelectric colorimeter, after clearing with ammonium hydroxide, at 540, 560 and 620 $\mu\mu$. Turbidity was especially troublesome in samples from gut, kidney, liver and lungs.

The spread of values obtained by hemoglobin extraction was much greater than that obtained with Fe[55] measurements. This is undoubtedly due to the inaccuracy of the former method. There is a very close agreement between the unit values obtained by both techniques, so that the measurement of total cells by Fe[55] radioactivity values in 2 experiments appears to have been warranted.

Scrutiny of data in Table IV reveals several points of interest. At once striking is the very small quantity of both cells and plasma per gram of tissue in all organs. The largest unit value of whole blood was found in the spleen, about 400 cu. mm. per gram. Liver, lungs, and kidneys contained about ½ as much, and heart and gastrointestinal tract about ⅐ as much as the spleen. Skeletal muscle and the brain contained very small quantities of whole blood.

A total of 17 per cent of the entire whole blood volume was found in the minute vessels of all the organs. This is about the figure given by authors who have computed the volume of the capillary bed under normal conditions (10). A somewhat greater proportion of the total plasma volume and a somewhat smaller proportion of the total red cells was in the minute vessels.

The greatest amount of whole blood was found in the liver, and in the skeletal muscle, from 5 to 6 per cent. From 1 to 2 per cent was found in

TABLE IV

Range of values for intravascular content of cells, plasma and whole blood in small vessels, and in large vessels; the hematocrits of blood in small and large vessels, the right auricle; the hematocrit of all the circulating blood; and the ratio of red cells circulating through the small vessels to the total red cell content of the small vessels

Findings in 7 normal dogs under light morphine narcosis

Organ	Percentage of body weight	Intravascular content of small vessels in ml./per gram of tissue						Per cent of total circulating volume						Hematocrit of blood in small vessels		Ratio of rapidly circulating to total cells
		Red cells		Plasma		Whole blood		Red cells		Plasma		Whole blood				
		Range	Av.	Range	Av.	Range	Av.	Range	Av.	Range	Av.	Range	Av.	Range	Av.	
														Per cent	Per cent	
Spleen	0.4	.18 to .56	.367	.05 to .15	.065	.23 to .55	.420	3.4 to 6.9	5.1	0.3 to 0.7	0.4	1.0 to 3.2	1.5	55 to 100	82	0.97
Liver	2.7	.02 to .08	.048	.06 to .16	.115	.12 to .23	.200	1.3 to 6.7	4.7	3.5 to 6.2	5.2	4.0 to 5.8	4.9	14 to 58	41	1.06
Lungs	0.7	.02 to .09	.063	.09 to .20	.115	.16 to .22	.195	0.3 to 1.8	1.1	1.0 to 2.9	1.6	1.0 to 1.9	1.4	9 to 44	33	0.95
Kidneys	0.7	.02 to .06	.037	.07 to .26	.174	.08 to .32	.205	0.1 to 0.9	0.5	0.6 to 2.2	1.7	0.4 to 1.6	1.2	9 to 22	15	0.99
Heart	0.8	.01 to .05	.022	.04 to .07	.049	.06 to .08	.066	0.2 to 0.4	0.3	0.3 to 0.7	0.6	0.3 to 0.6	0.5	17 to 28	22	1.03
Bowel	3.5	.004 to .015	.006	.04 to .07	.050	.04 to .07	.060	0.5 to 2.3	1.0	1.8 to 4.7	2.9	1.2 to 2.8	1.5	8 to 30	17	1.05
Muscle	33.3*	.002 to .007	.004	.01 to .02	.014	.01 to .02	.018	2.0 to 4.7	3.4	3.4 to 16.6	7.5	3.3 to 10.9	5.8	8 to 32	21	1.04
Brain	0.6	.003 to .01	.006	.01	.01	.01	.013	0.3 to 0.4	0.4	0.1 to 0.9	0.5	0.1 to 0.7	0.4	16 to 21	18	
Total	42.7								16.5		20.4		17.2			

Weighted average: Ratio of rapidly circulating to total cells = 1.01 ±0.05

Average hematocrit of auricular blood. Range from 33.7 to 50.0. — { 29.8 / 23.2† }

Average hematocrit of all circulating blood in large vessels. Range from 32.9 to 47.3. — 43.3

Average hematocrit of all circulating blood. Range from 31.9 to 44.3. — 40.2 / 36.8

* Estimated from values in 2 dogs in which all muscle removed from skeleton.
† Excluding splenic blood.

TABLE V

The hematocrits of whole blood in large and minute vessels and of the whole body compared to the arterial hematocrit

Experiment no.	111	113	114*	127	131	Av.	Ratio to arterial hct.
Arterial or venous	44.6	46.7	50.0	33.7	42.3	43.3	1.0
Large vessels	33.9	46.0	47.3	32.9	40.6	40.2	0.92
Body	31.9	43.0	44.3	32.0	40.2	36.8	0.85
Minute vessels	22.0	34.2		25.9	37.6	29.8	0.68

* Splenectomized dog.

spleen, lungs, kidneys and gut, while brain and heart contained less than 1 per cent.

While a very small portion of total whole blood volume was found in the spleen, this amount is a sizeable proportion, from 1/10 to 1/5 of the total whole blood in the minute vessels.

The hematocrit of whole blood in each organ varied greatly in individual dogs, by as much as a factor of 4. The average values given in Table IV are weighted. There were marked differences in these values for the several organs. The he-

matocrit of splenic blood was higher than that of any other organ, and also higher than that of arterial or venous blood. Liver blood had an hematocrit about equal to arterial blood, while pulmonary blood had a definitely lower hematocrit. The hematocrits of blood in all the other organs is about ½ that of arterial blood.

Thus it is apparent that the cell to plasma ratio of a great part of blood in capillaries is very low. It is quite possible that this low ratio is in some way related to the speed of flow through various organs, and to the larger intimal surface area of capillaries as compared to artery. Circulation in the spleen, which had the highest hematocrit value, is known to be sluggish, while flow through kidney and heart, which had the lowest hematocrit values, is far more rapid. Liver and spleen have sinusoidal vessels which are of larger calibre than capillaries, and therefore have proportionately less surface that must be wetted by plasma.

The hematocrit of all the blood in the body was lower than the arterial hematocrit in all 5 dogs, by an average value of 85 per cent. The minute
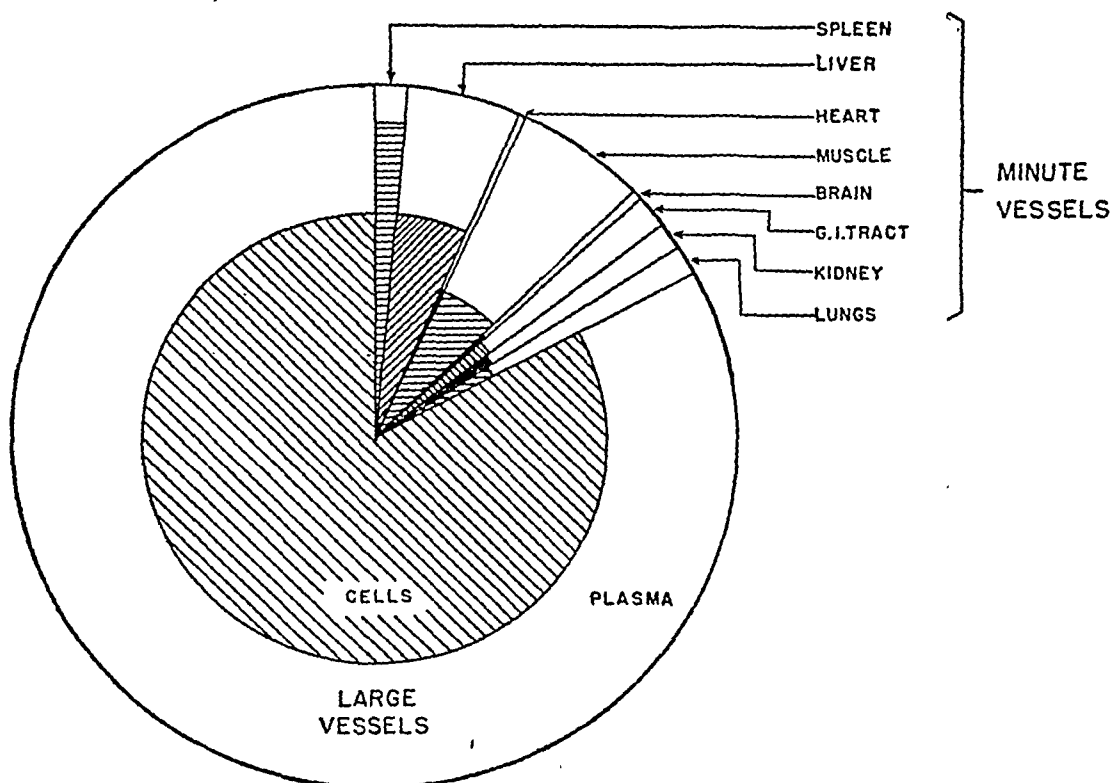
FIG. 2. THE RELATIVE DISTRIBUTION OF WHOLE BLOOD, CELLS AND PLASMA IN THE LARGE AND MINUTE VESSELS

The hematocrits of whole blood in the large vessels, and in the several organs are indicated by the segments of the inner circles.

vessel hematocrit was considerably lower and the large vessel hematocrit slightly higher than the body hematocrit. Figure 2 shows the relative distribution of whole blood and the relative concentration of red cells, in both the large and minute vessels.

In a previous communication (1) we reported the ratio of red cell volume measured simultaneously by radio-iron and dye averaged 0.85 in 40 normal males, and 0.83 in 40 normal dogs. As shown in Table V, this ratio, in 4 dogs in which all organs including the spleen were studied, averaged 0.85. It was of interest to ascertain what corresponding ratio would be obtained when the calculation thereof was based on the quantities of red cells and plasma in minute vessels, determined directly by radio-iron and radio-iodine measurements. This analysis is given in Table VI. To determine red cell volume, the percentage of whole blood in the minute vessels is multiplied by the ratio of the hematocrit of that blood. Large vessel blood is considered as the difference between total blood volume measured by dye and radio-iron, and the minute vessel blood. The percentage of whole blood in the large vessels is also multiplied by the ratio of the hematocrit of that blood,

and the 2 products are added. For example,

Whole blood in large vessels = 82.8 per cent of total blood.

Whole blood in minute vessels = 17.2 per cent of total blood.

The ratio of hematocrit of large vessel blood and of minute vessel blood to arterial hematocrit are 0.92 and 0.68 respectively.

Then,

$$.828 \times 0.92 = .763$$
$$.172 \times 0.68 = \underline{.117}$$
$$.880$$
$$= Vrr/Vrpd \text{ or } Vrpd/Vrr = 1.14$$

There was fairly good agreement between this value and the value for Vrr/Vrpd based on gross volume determinations in all experiments. The ratio based on minute vessel measurements averaged 0.88, that based on gross volume measurements averaged 0.85. This agreement constitutes an excellent internal check on the accuracy of all tissue measurements.

These observations fully explain why Vrr is consistently 85 per cent of Vrpd in the normal state.

TABLE VI

Relationship of whole body to arterial hematocrit based on direct radioactivity measurements of cells and plasma in minute vessels, compared to the same ratio obtained by gross radio-iron red cell volume measurements

	Experiment no.*				Averages
	111	113	127	131	
Calculations based on direct radio-iron and radio-iodine analyses					
Fraction of whole blood in minute vessels	0.172	0.171	0.152	0.162	0.164
Ratio of minute vessels to arterial Hct.	0.54	0.77	0.78	0.61	0.675
Whole blood x ratio of hematocrits	0.093	0.133	0.12	0.10	0.112
Fraction of whole blood in large vessels	0.828	0.829	0.848	0.838	0.836
Ratio of large vessel to arterial Hct.	0.76	0.98	0.99	0.93	0.915
Whole blood x ratio of hematocrits	0.63	0.81	0.85	0.78	0.768
Sum of whole blood in minute and large vessels x ratio of hematocrits	0.72	0.94	0.97	0.88	0.880
Calculations based on gross red cell (by radio-iron) and plasma volumes (by dye method)					
	ml.	ml.	ml.	ml.	
Red cell volume by radio-iron	650	780	345	463	
Plasma volume by dye method	1390	945	740	690	
Whole blood volume by dye method	2510	1770	1100	1220	
Red cell volume by dye method	1120	825	360	530	
Ratio of Vrr to Vrpd	0.58	0.95	0.96	0.88	0.842

* Experiment No. 114, splenectomized dog, is excluded.

Since Vrpd is always calculated from the plasma volume and auricular arterial or venous hematocrit, it follows that true red cell volume is also over-estimated by about 15 per cent by the dye method. The agreement of the above value, 0.88, with the values obtained by gross volume measurements of 0.85 for normal humans and 0.83 for normal dogs, is convincing evidence, in 2 independent sets of experiments, in support of the above statement.

The ratio of rapidly circulating to total red cells is a measure of the completeness with which the second infusion of tagged red cells has become mixed with all the red cells within the entire vascular bed. If this ratio is 1.0, it indicates complete mixing. If it is less than 1.0, it indicates that some fraction of the total red cells are no longer in active circulation. A ratio greater than 1.0 is theoretically impossible.

The ratios for the several organs varied from 0.95 to 1.06, averaging 1.01 ± 0.05. Thus all values are well within the limit of error of the techniques employed. We conclude that in the normal dog all of the red cells within the vascular bed are in active circulation. It follows that no reserves of erythrocytes exist in the body of the dog in the organs studied.

These studies do not disclose what quantities of mature cells may be in bone marrow. Since the normal rate of erythrocyte production is only 1 per cent of total red cell volume per day, it is extremely doubtful if bone marrow can be considered as a reserve of any significance. These facts invalidate the hypotheses of Wollheim (11), Levin (12) and Goldbloom and Lieberson (13), who considered the so-called "blood depots" of such a magnitude as to contain an amount of red cells almost equal to the quantity in active circulation.

The spleen has been thought of as a great blood depot. Contraction of that organ through sympathetic or adrenalin stimulation was thought of as flushing a great number of red cells into the vascular bed. This contention seemed to be supported by an increase in the hematocrit of venous blood following the administration of adrenalin, which was considered as evidence of an increase in total circulating red cell volume (14). Hahn (15) has shown that while this rise in hematocrit is produced by adrenalin in dogs, there is no corresponding change in the circulating red cell mass as directly measured by means of infused red cells tagged with radio-iron, and suggests that the rise in hematocrit may be due to redistribution of red cells brought about by vasoconstriction.

The total amount of red cells found in the spleen in these experiments was less than 5 per cent of the total red cell volume. These animals were sacrificed, and there is the possibility that a terminal contraction of the spleen, which would have emptied the sinusoids of some of their red cell content, may have occurred. However, the spleens were soft and did not appear to be contracted. Hahn has shown (16) that nembutal in anesthetic doses may cause the spleen to relax and become engorged with blood. It does not follow that a similar reaction is induced by sudden massive lethal doses of nembutal.

The experiments described were an attempt to study one aspect of the dynamics of the circulation. It should be emphasized that the volume techniques employed measure only plasma and red cells in *active circulation*. Therefore the values obtained are an expression of the amount of blood flowing through the organs at a given time.

The content of plasma and red cells found in these studies represents the status in the normal dog. This relationship may well change in abnormal circulatory conditions, as a result of extreme vasoconstriction or vasodilation. These studies present no data on the expansibility or contractability of the minute vessel plexus.

The most striking finding is the extremely small fraction of the total blood mass that is actually flowing through the nutrient vessels of the body at any one time. Only $\frac{1}{5}$ of the blood is performing metabolic work at any one time, the remaining $\frac{4}{5}$ being in transit to and from the site of cellular life processes and the sources of oxygen and food supply. The extent to which this may be thrown out of adjustment by experimental procedures which induce peripheral vascular collapse will be reported in subsequent communications.

CONCLUSIONS

1. Seventeen per cent of the total whole blood volume is within the minute vessels (arterioles, capillaries and venules) of the normal dog under light morphine narcosis.

2. The unit values of whole blood (ml. per gram of tissue) of the various tissues range in order of

magnitude as follows: spleen 0.4 ml.; liver, lungs and kidneys 0.2 ml.; heart and gastrointestinal tract 0.06 ml.; and skeletal muscle and brain 0.02 ml.

3. The percentage of total whole blood volume in the various organs is: skeletal muscle, 7 per cent; liver, 5 per cent; spleen, lungs, kidneys, gastrointestinal tract (excluding mesentery), from 1 to 2 per cent each; heart and brain, less than 1 per cent each.

4. The hematocrit of blood in the several organs, in animals with an average arterial hematocrit of 43 are approximately: spleen, 80; liver, 40; lungs, 35; heart and skeletal muscle, 20 to 25; and kidney, gastrointestinal tract and brain, 15 to 20.

5. The hematocrit of the blood in the large vessels, of all the blood in the body, and of the blood in the minute vessels, is always less than that of arterial or venous blood. The ratios of the partition hematocrits to the arterial hematocrit are approximately 0.9, 0.85 and 0.7 respectively.

6. The ratio of rapidly circulating to total red cells in the several organs is unity. Hence all red cells in the vascular bed are in active circulation, and no reserves of red cells exist in the normal dog.

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COMPARISON OF THE EFFECTS OF MASSIVE BLOOD TRANSFUSIONS AND OF LIVER EXTRACT IN PERNICIOUS ANEMIA [1]

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The features of the classical, clinical and hematological response to the administration of liver (1) and of stomach (2) preparations, and recently of synthetic *L. casei* factor (3, 4) in patients with untreated pernicious anemia, are well known. The action of these therapeutic agents is, however, sometimes rather carelessly regarded as one of direct stimulation of blood production. It is perhaps more accurate to consider that liver extract acts by the replacement of a nutritional deficiency and so, in one way or another, permits effective response of the bone marrow to the stimulus of the anoxia common to all anemias. Moreover, despite an obvious analogy with the effects of iron administration in patients with hypochromic (iron deficiency) anemia, the concept that liver extract therapy in pernicious anemia also acts by abolishing a maturation arrest leading to decreased blood production has recently been challenged (5 to 7). This represents a return to the older viewpoint that the anemia is based on excessive blood destruction, manifest especially by the increased output of bile pigments in the feces. Under this hypothesis, the nutritional deficiency is assumed in some way to permit the advent of a hemolytic process (7), and the absence of elevated reticulocyte counts in the peripheral blood is explained on the hypothesis that these cells are destroyed selectively before they leave the bone marrow (5). The megaloblastic hyperplasia of that organ is thus interpreted as an unusually severe degree of the erythroid cell immaturity seen in the bone marrows of severe hemolytic anemias in animals (8) and in man (9, 10). It appeared to be desirable to secure, if possible, additional information on some of these points of view, and to attempt to distinguish between the direct effects of liver extract therapy and those due to elevations in hemoglobin levels following its use. Accordingly, observations were made of the comparative hematological and clinical effects of rapidly repeated transfusions, and of the administration of liver extract in pernicious anemia.

METHODS

Five patients with classical Addisonian pernicious anemia in relapse were selected, and were given daily transfusions of whole blood or red cell concentrates until, in 2, the red cell counts reached 3 million per cu. mm., and in 3, the red cell counts reached 5 million per cm. mm. Liver extract was then administered to the 2 patients with red cell counts of 3 million per cu. mm., and to 2 of the 3 patients with 5 million red cells per cu. mm. The remaining patient with a normal red cell value was given no further therapy for 46 days, when his red cell count had fallen to 3 million per cu. mm. Liver extract was then administered. Both during the period of transfusion, and after liver extract administration, the changes in clinical condition were observed, as well as changes in red cell count, hemoglobin concentration, corpuscular indices, reticulocytes, white cell and platelet counts, and icterus index. Sternal marrow puncture and biopsy were performed at appropriate times in order to determine the comparative effects on the marrow cytology of the red cell transfusions, and of the administration of liver extract.

The blood cell counts were made on oxalated venous blood with U. S. Bureau of Standards pipettes and counting chambers. The hemoglobin was determined using the Klett-Summerson colorimeter, using 15.6 grams of hemoglobin per 100 ml. of blood as the standard. The red cell indices were obtained by the method of Wintrobe (11). Blood platelet counts were performed by a modification of the method of Rees and Ecker (12). The icterus index was determined by comparing plasma with dichromate standards (13). Bone marrow puncture was made in the mid-sternum at the level of the second interspace, using the needle designed by Turkel (14). Sternal marrow biopsies were done by the surgical service, using a ¼-inch trephine and standard surgical technique.

Whole blood for transfusions was collected in sodium citrate solution by the hospital blood bank. Red cell concentrates were prepared by centrifuging fresh citrated blood and withdrawing most of the plasma. A small amount of saline (50 to 75 ml.) was added and well

mixed with the cells before infusion. Cell concentrates were used before they were more than 3 days old.

RESULTS

(a) Red blood cell values and indices.

During the period of transfusions, there was a rapid rise of the red cell count, hemoglobin concentration and hematocrit.

Case No. 1 received the red cell equivalent of 9,750 ml. of blood, 7,900 ml. of which were necessary to bring the red cell values to normal in 13 days. Case No. 2 received the red cell equivalent of 6,500 ml. of blood, all as red cell concentrates,

bringing the red cell values to normal in 7 days. Cases No. 3 and No. 4 received the red cell equivalent of 3,000 ml. of blood in 3 days, with a resultant red cell count of about 3 million per cu. mm. Cases No. 5 received the red cell equivalent of 8,000 ml. of whole blood in 8 days, resulting in an essentially normal red cell value.

(b) Reticulocytes.

During the period of blood transfusions there was no significant rise in the reticulocyte count in any of the patients.

In the 2 patients given multiple U.S.P. units of liver extract by intramuscular injections immedi-
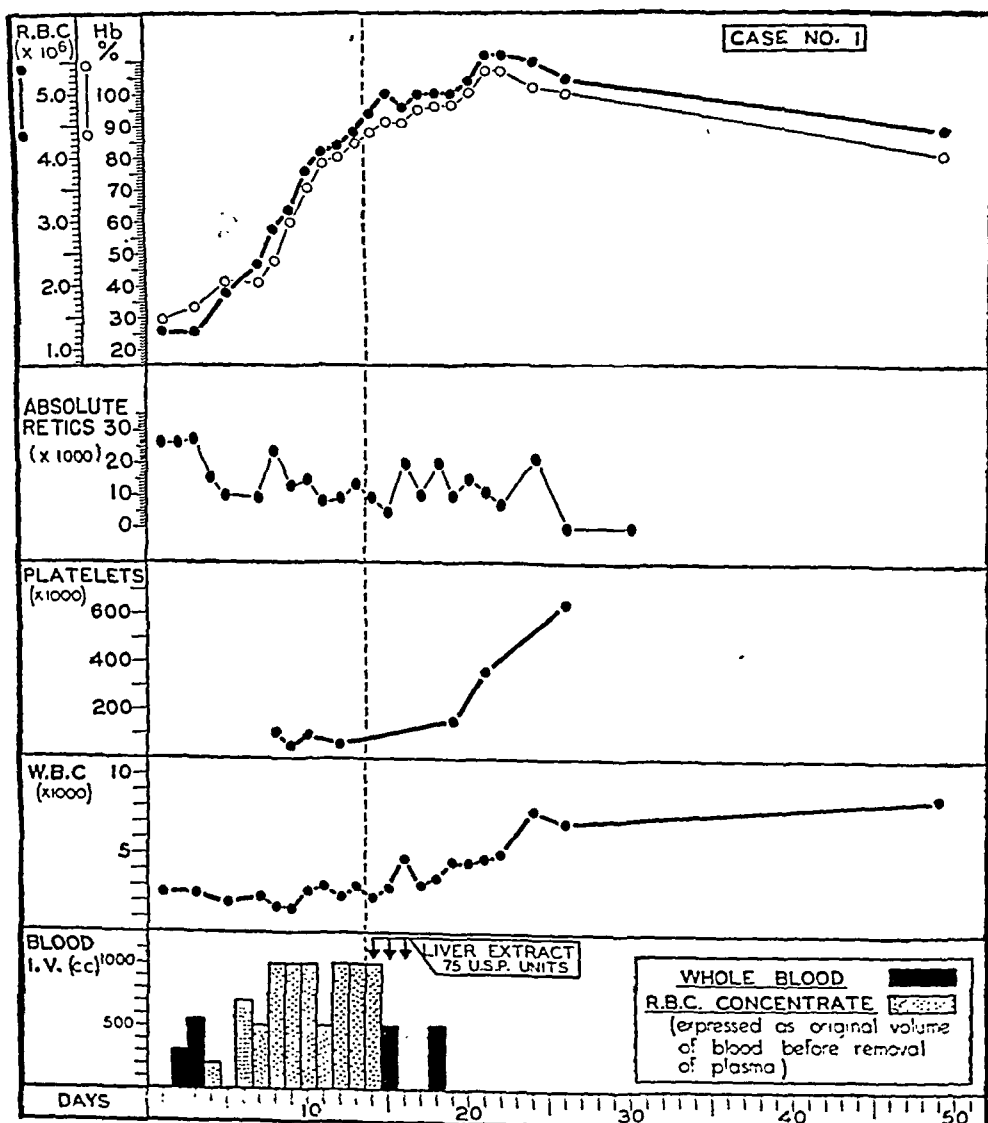
FIG. 1. CASE No. 1

ately after the end of the series of transfusions when their red cell values were normal, no rise in reticulocytes occurred in Case No. 1 (Figure 1), and only a slight rise, reaching 60,000 per cu. mm., in Case No. 2. The peak of this response was on the 7th day after liver extract was first injected, and after the red cell count had fallen to about 4 million per cu. mm. (Figure 2).

The 3 patients given liver extract when their red cell counts were near 3 million per cu. mm.
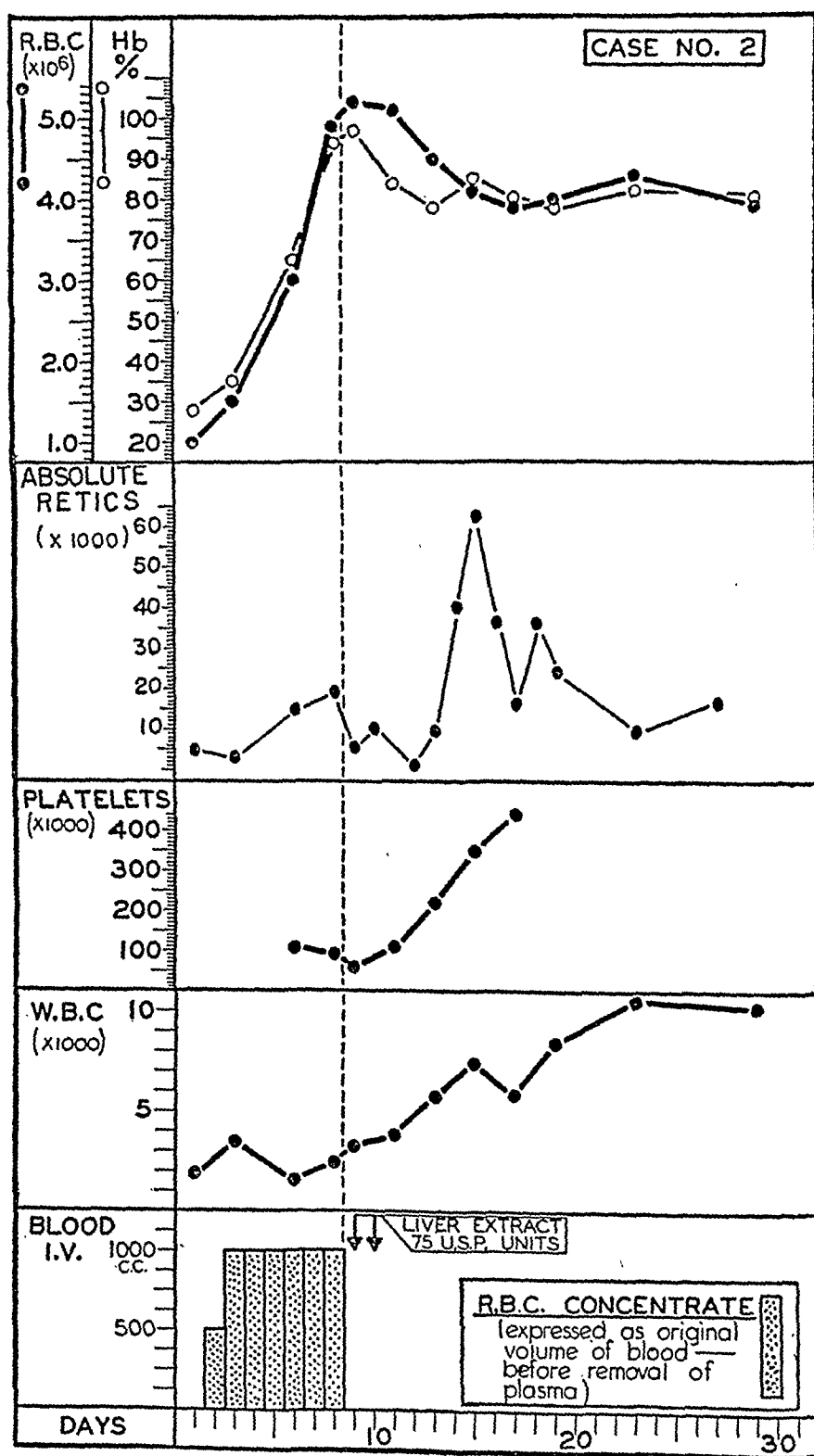
FIG. 2. CASE No. 2
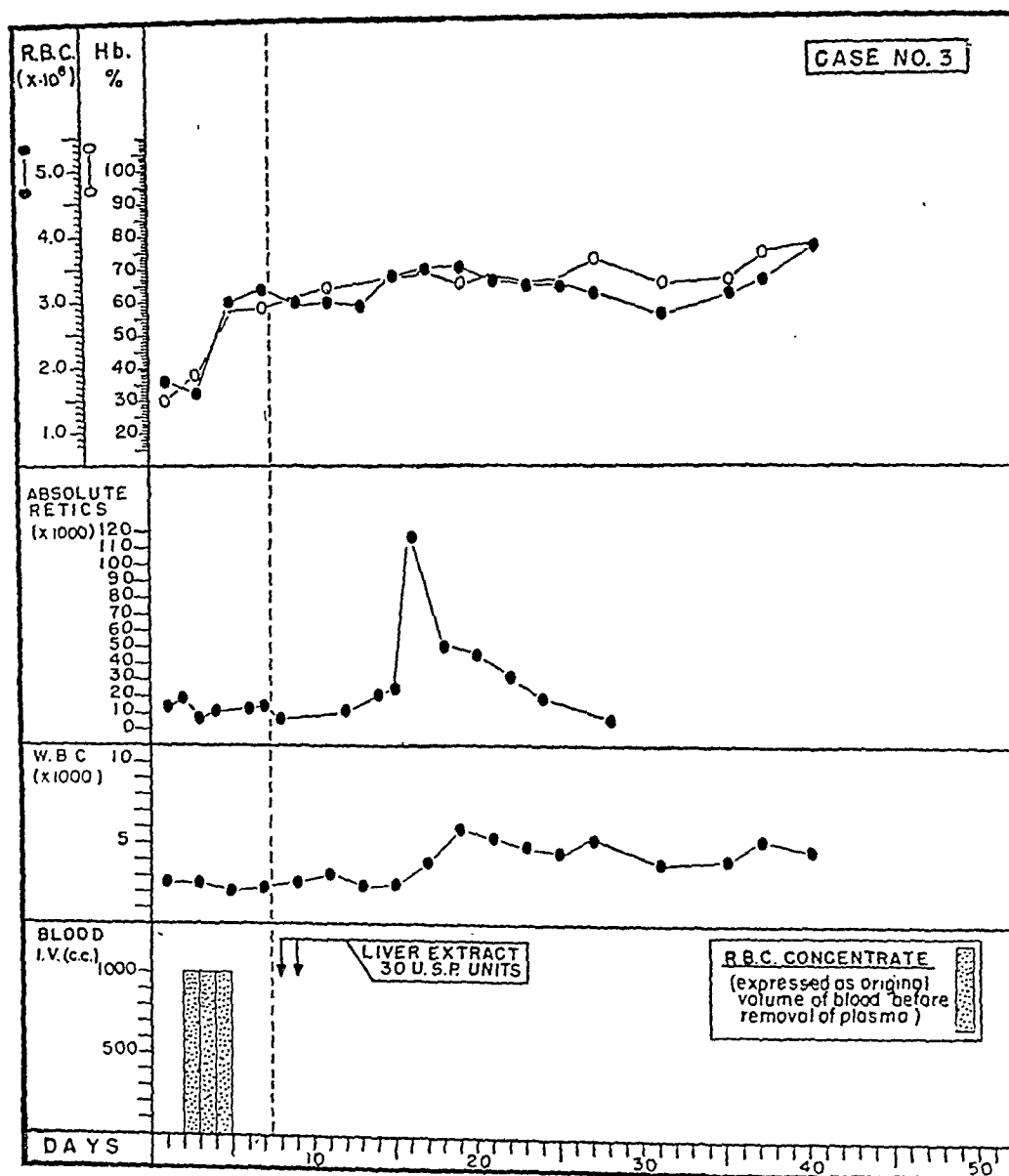
Fig. 3. Case No. 3

had rises in reticulocyte counts reaching maxima of 123,000, 116,000 and 326,000 per cu. mm., respectively, in Cases Nos. 3, 4, and 5.

(c) *White blood cells and platelets.*

Leukopenia was present in all patients before and during the period of blood transfusions. In fact, it was only after the administration of liver extract that a significant rise in the white cell count occurred. That this rise was not due to a delayed response to previous transfusions is made clear by the observations in Case No. 5 (Figure 5),

in which a period of 55 days separates the transfusions from the administration of liver extract.

Thrombocytopenia was still evident during the transfusions in the 4 patients in whom platelet counts were made. Here again, it is evident that no significant rise in blood platelets occurred until after liver extract was administered. This is particularly well shown in Cases Nos. 1, 2, and 5 (Figures 1, 2, 5), and is suggested in Case No. 4 (Figure 4), in whom but 2 platelet counts were made.

(d) Bone marrow.

A summary of the differential bone marrow nucleated cell counts is presented in Table I. The polymorphonuclear and band form granulocytes remained about the same throughout, except in Cases Nos. 1 and 2 where a definite increase was seen after liver extract therapy.

More striking, however, is the change in megaloblast percentage following blood transfusions. No examination of the bone marrow was made in Case No. 1 before transfusions were begun, but undoubtedly megaloblasts were present in the quantity usual for pernicious anemia in relapse. Following transfusions they almost disappeared. The effect of transfusions in sharply decreasing the percentages of megaloblasts is seen in the differential counts of marrow cells obtained from Cases Nos. 2, 3, and 5, and is illustrated by the photomicrographs (Figures 6, 7). Furthermore, in Case No. 5, the megaloblasts reappeared during the 1½ months after transfusions were discontinued, at the end of which time the red cell
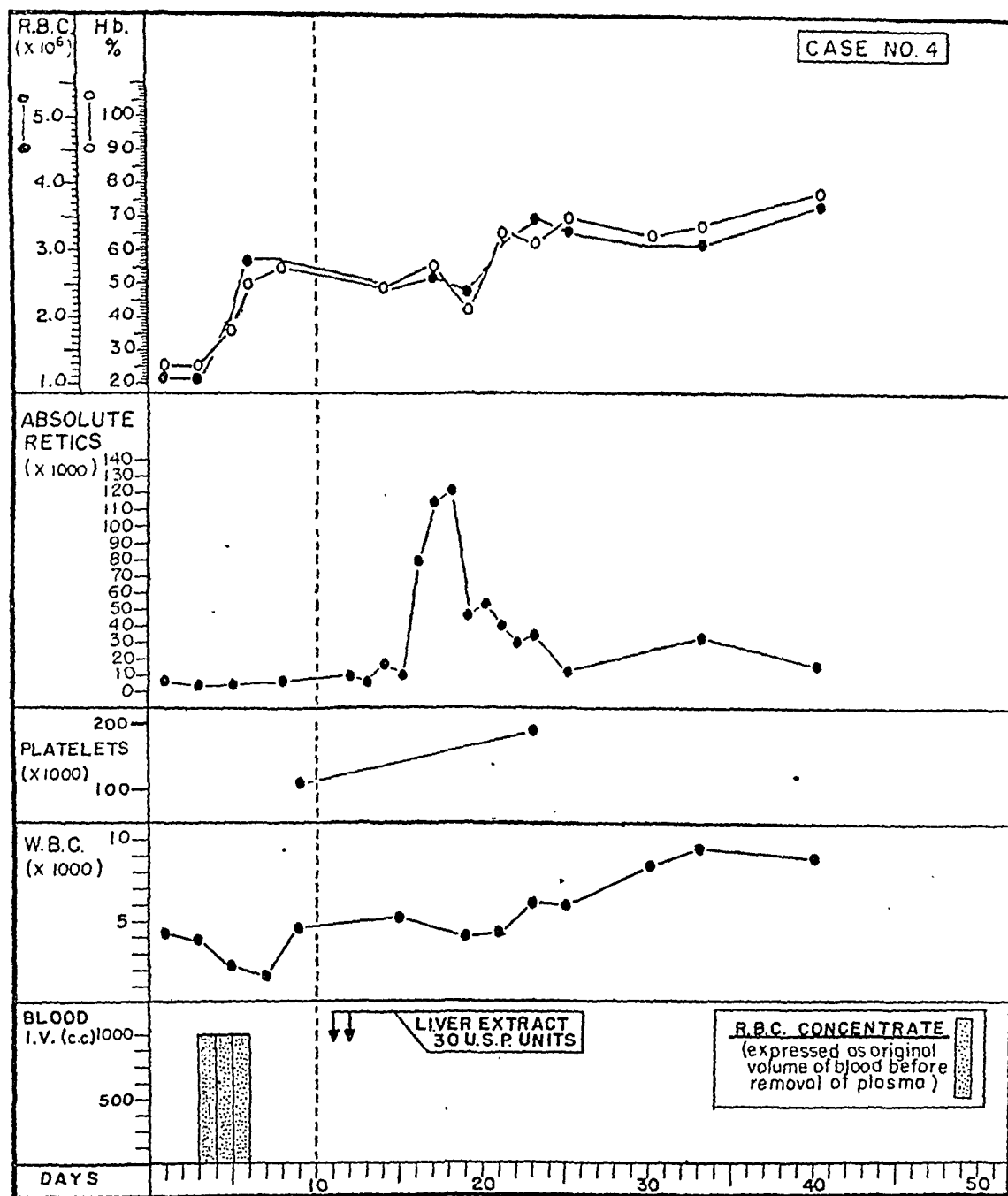
Fig. 4. Case No. 4
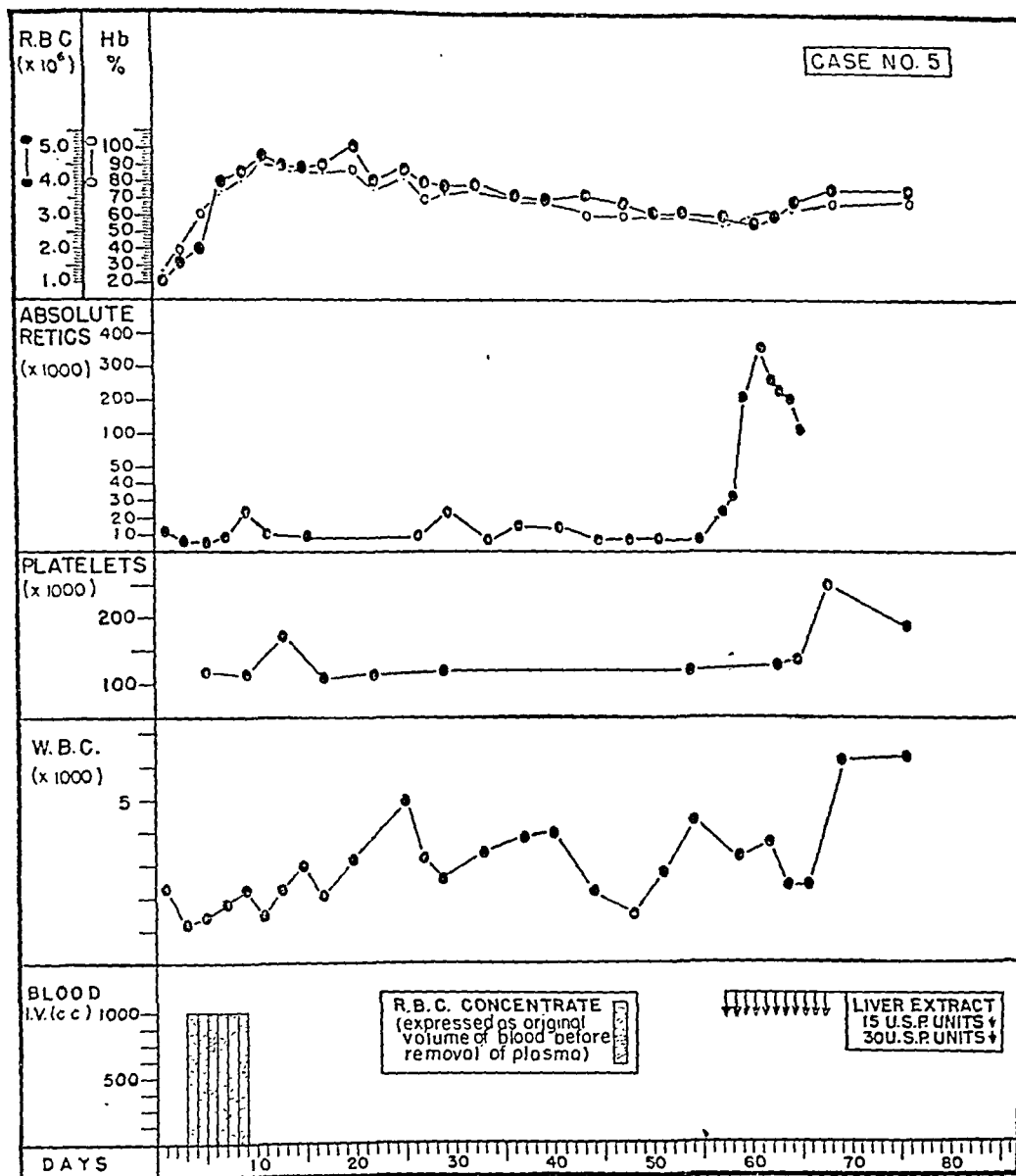
FIG. 5. CASE No. 5

count had dropped from 5 to 3 million per cu. mm.

(e) Clinical course.

Case No. 1, a 67-year-old white female, known to have had pernicious anemia in the past, suffered a relapse from failure to continue liver extract therapy. Before transfusions were begun she complained of weakness, anorexia, "indigestion," and a recurrence of nervous system symptoms. Inspection showed marked pallor, white hair, blue eyes, and a pale, smooth tongue. There was cardiac enlargement and dependent edema. Drowsiness with euphoria were the chief mental symptoms.

During the course of transfusions, the patient became even less active and responsive, and dozed most of the time. Her tongue became fiery red, with fissures, but no growth of papillae became evident. Her pallor was, however, replaced by normal skin color. This effect, which was presumably due simply to the return to normal hemoglobin concentration, was the only sign of clinical improvement on the 14th day, when liver extract was administered. On the 21st day, 7 days after liver extract was begun, the patient seemed for the first time definitely better. She appeared more alert, and took a more active interest in her surroundings. This improvement progressed rapidly, so that a week later she was up in a chair most of the day. The tongue had become less red, and papillae were beginning to appear. There was no significant change in

symptoms or signs of spinal cord disease during the period of observation.

Case No. 2, a 45-year-old white male, was admitted in his first attack complaining of fatigue and weakness of several months' duration. He had had constipation and attacks of "indigestion," together with progressive anorexia. He had noticed numbness and tingling of his finger tips for about 1 week He was poorly nourished and pale; there was moderate loss of papillae on the tongue, especially along the margins. A definitely impaired vibration sense was present in both lower extremities. Mentally he was clear but a little lethargic. His pallor was replaced by normal color during the period of transfusion. However, although ambulatory since admission, his lethargy

continued, and anorexia, "indigestion," and tongue changes likewise remained the same.

Beginning about 7 to 10 days after the administration of liver extract, there was a distinct change in the patient's condition. He became brighter and more cheerful. His appetite improved rapidly, and the tongue showed some regrowth of papillae. His paresthesiae disappeared in the course of about 2 weeks.

Case No. 3, a 59-year-old white female, in whom a diagnosis of pernicious anemia had been made 2 years previously, suffered a relapse after discontinuing liver therapy. Before blood transfusions were initiated, she complained of weakness, "indigestion," anorexia, and "gas" after meals. She noticed pounding in the head and had palpita-

TABLE I

Distribution of nucleated bone marrow cells

Status	Day	Polymorphs. and bands	Metamyelocytes and myelocytes	Normoblasts	Erythroblasts	Megaloblasts
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
Case no. 1 After transfusion Before liver extract R.B.C. 4.70 million	14	22	36	6	8	3
35 days After liver extract R.B.C. 5.1 million	49	40	24	10	6	1
Case no. 2 Before transfusion R.B.C. 1.03 million	1	29	22	7	10	25
After transfusion Before liver extract R.B.C. 5.17 million	8	25	25	8	9	2
15 days After liver extract R.B.C. 4.30 million	23	44	20	6	7	0
Case no. 3 Before transfusion R.B.C. 1.18 million	3	16	18	11	15	28
After transfusion Before liver extract R.B.C. 3.17 million	7	29	12	11	8	1
6 days After liver extract R.B.C. 2.94 million	13	17	32	25	19	1
Case no. 5 Before transfusion R.B.C. 1.02 million	2	21	8	11	19	21
During transfusion R.B.C. 2.0 million	5	27	18	9	12	11
During transfusion R.B.C. 4.0 million	8	22	11	18	8	2
End of transfusion R.B.C. 4.5 million	12	11	40	9	14	2
No therapy 46 days R.B.C. 3.0 million	58	27	13	10	20	10
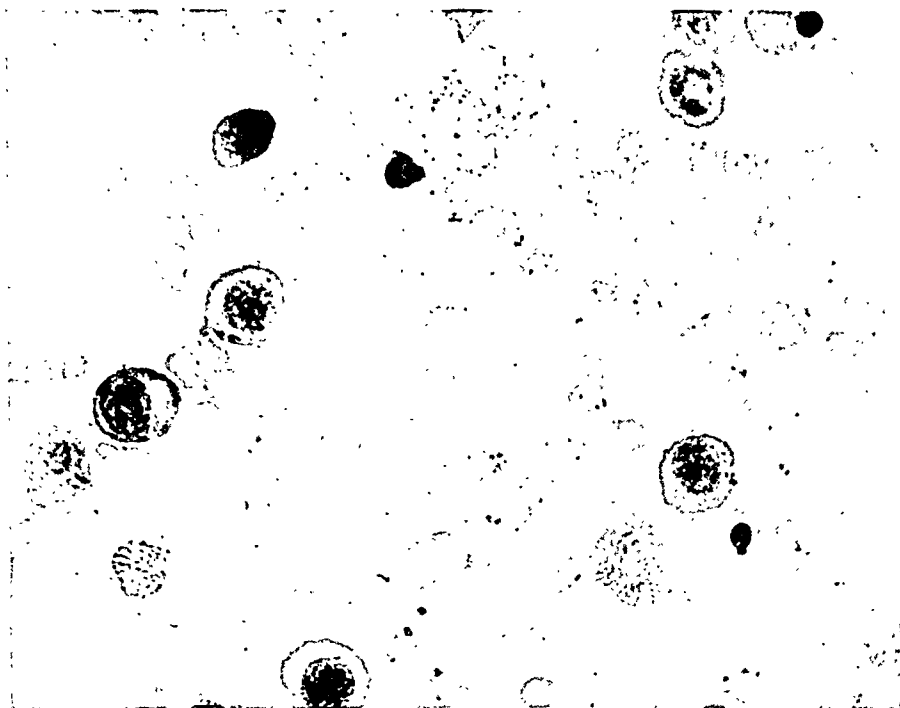
FIG. 6. PATIENT No. 2—DAY 1. BEFORE TRANSFUSION

Red cell count, 1.03 million per cu. mm. Bone marrow film with large proportion of megaloblasts. (Wright's stain, oil immersion.)
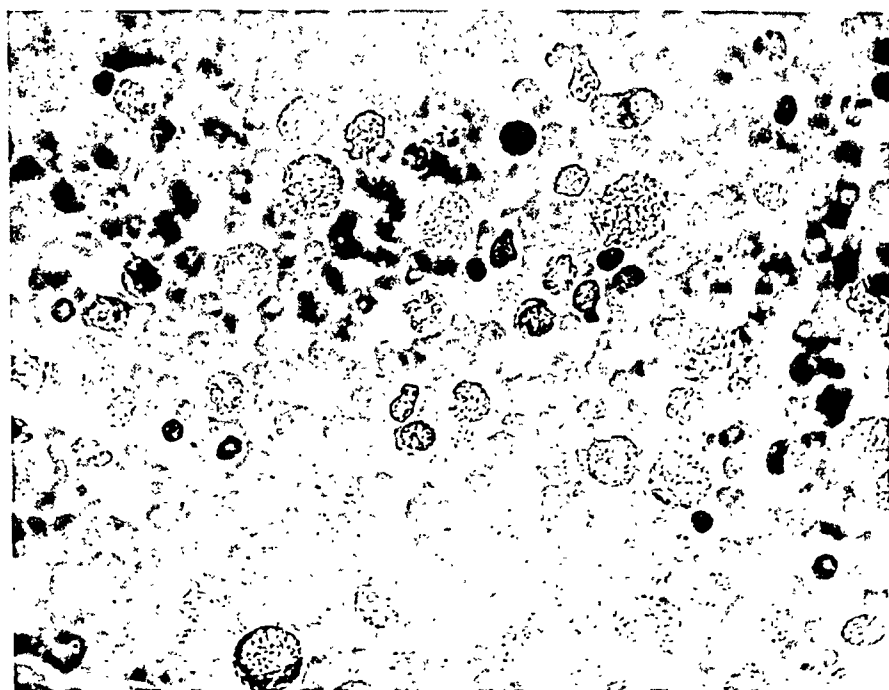
FIG. 7. PATIENT No. 2—DAY 8. TRANSFUSIONS FINISHED

Red cell count, 5.17 million per cu. mm. Bone marrow film is relatively normal. Megaloblasts gone. (Wright's stain, oil immersion.)

tion on exertion or on becoming disturbed emotionally. Her skin was waxy-yellow in appearance; the hair gray, the eyes blue, and the tongue papillae atrophic. Cardiac enlargement was moderate, but there was no dependent edema.

During and immediately after the course of red cell transfusions the patient noted disappearance of the head pounding and palpitation, and her color improved. There were no other subjective or objective changes. Soon after the reticulocyte peak, following liver extract administration, the patient began to improve markedly. Her appetite increased, gastro-intestinal symptoms diminished, and weakness gradually disappeared.

Case No. 4, a 73-year-old white male in whom a diagnosis of pernicious anemia had been made a year previously, later discontinued treatment with liver extract and was admitted after having collapsed. He confessed to having orthopnea and anginal pain on slight exertion. Marked pallor was evident on examination. The hair was gray, the eyes brown, and the tongue papillae moderately atrophic. No cardiac enlargement was observed, but dependent edema was present. The spleen was palpable at the left costal margin. There was no evidence of combined system disease.

Following red cell transfusions, orthopnea and anginal pain entirely disappeared, but anorexia and a listless facial expression continued. Following the administration of liver extract these also gradually disappeared, although not as dramatically as in the foregoing 3 patients.

Case No. 5, a 75-year-old white male without a previous history of pernicious anemia, on admission complained of increasing weakness, anorexia, and sore tongue of about 1 month's duration. On examination he was found to be markedly pale and emaciated, with an atrophic tongue. There was no cardiac enlargement nor dependent edema. No evidence of combined system disease was observed except for diminished vibratory sense in the lower extremities.

Following transfusions, his weakness decreased somewhat, but, in general, he was symptomatically the same for the 1½ months between blood transfusions and liver extract therapy. However, following the latter there was striking subjective and objective improvement. Especially remarkable was the regrowth of papillae on the tongue.

DISCUSSION

The administration of liver extract, in a decisively effective dose, to patients with pernicious anemia in relapse, produces certain constant physiological effects. After as little as 6 hours, the primitive megaloblasts of the bone marrow begin to decrease while a corresponding increase in the more mature cells takes place (15). Within 3 to 4 days the predominant erythroid cell is the normoblast. At about this time, the characteristic reticulocyte rise begins in the peripheral blood, and relief of anorexia with increase in strength

and sense of well-being are experienced by the patient. The leukocytes, and especially the platelets, also increase in the peripheral blood. It is noteworthy that clinical improvement is often striking before any significant increase occurs in the levels of red cells and hemoglobin.

In none of the cases reported here, however, did transfusions modify the blood findings or clinical status, other than to abolish the anemia and the symptoms directly related to decreased hemoglobin concentration in the peripheral blood: pallor, palpitation, or angina. Anorexia, apathy, and digestive symptoms were not affected. Transfusions also failed to cause any increase in reticulocytes, or in leukocytes or platelets.

Although, in contrast to transfusions, liver extract therapy apparently caused remarkable clinical improvement and increases in leukocytes and platelets, the response of the reticulocytes was greatly modified by previous transfusions. Thus, reticulocyte response to liver extract therapy was absent in Case No. 1 with 5 million red cells per cu. mm., was slight in Case No. 2, whose red cells had dropped to about 4 million per cu. mm., was moderate in Cases No. 3 and 4 which were transfused to only 3 million per cu. mm., and was marked in Case No. 5, in which the red cells were allowed to drop from 5 to 3 million per cu. mm. Thus, the reticulocyte response to liver extract was roughly inversely proportionate to the height of the artificially elevated red cell count and hemoglobin present at the time of its occurrence, and not to their low values before transfusions were given.

Such depression, or even failure, of the reticulocyte response to occur after liver extract therapy at artificially elevated red cell and hemoglobin levels could theoretically be the result of at least 2 effects of the transfusions. First, the abolition of the anemia, and consequently of the general accepted stimulus to red cell production, anemic anoxia. Second, the introduction of substances in the transfused normal blood equivalent in physiological action to liver extract, with resulting partial or complete inability of the patient to respond to a subsequent injection of liver extract.

Experiments on animals as well as observations in man have shown that the number of reticulocytes in the peripheral blood becomes decreased when the tissue oxygen tension is increased either

by transfusions or by breathing high concentrations of oxygen (16 to 18). In pernicious anemia in relapse, the inverse relationship between height of reticulocyte response to liver extract and initial red cell (or hemoglobin) level (19) is well known. For red cell levels above 3.5 million per cu. mm., reticulocyte responses are slight or absent. This diminished response is conceivably due either to the relatively slight deficiency of the active principle of liver extract presumably existing in patients with such relatively high blood levels, or to the comparatively slight degree of anemic anoxia of their bone marrow. The present observations, in which for reasons given below it is probable that the existing deficiency of liver extract was not modified by the previous transfusions, suggest the latter explanation as the correct one.

As already indicated, the bone marrow was found to be strikingly altered in its morphology by transfusions given prior to the administration of liver extract. The percentage of typical megaloblasts in the bone marrow became sharply reduced within from 4 to 12 days after transfusions were begun, as the red cell counts rose in the peripheral blood. Because this effect is also so characteristic a sequel of the administration of liver extract, it is necessary to consider whether the transfusions of normal blood could have supplied, or have been equivalent to, the active principle of liver extract in these patients.

In numerous observations on patients with pernicious anemia, there has never been, in our experience, any detectable elevation of reticulocyte levels in the peripheral blood from transfusions of 500, or sometimes 1,000 ml. of whole blood during control periods without other therapy. The negative effect of even larger transfusions upon reticulocytes is also convincingly shown in Cases Nos. 3 and 4 in which responses to the administration of liver extract followed shortly. This evidence, together with the failure of the white cells and platelets to increase, and especially the lack of significant clinical improvement, are in sharp contrast to the striking effects subsequently observed when liver extract was administered.

At first thought, it seems remarkable that the characteristic appearance of the bone marrow in pernicious anemia, which, since Peabody's (20) work, has been interpreted by many observers as evidence of a specific "maturation arrest," should

be susceptible to rapid modification by transfusion alone. However, it is unlikely that all adult red cell production is at a standstill in untreated pernicious anemia. Consequently, if it is assumed that the megaloblasts are the precursors of more mature erythroblasts, and finally of adult reticulocytes and adult erythrocytes, the so-called "maturation arrest" must be a relative rather than an absolute state. Indeed, it has been suggested that the extensive, highly cellular and erythropoietically immature bone marrow picture in pernicious anemia is simply the response of that organ to the anemia resulting from a hemolytic process which destroys erythrocytes, especially reticulocytes, before they leave the marrow (5). Certainly the greatly increased output of urobilinogen (21, 22) and of coproporphyrin Type I (7) in the stools find their analogy in the classical hemolytic anemias, such as congenital hemolytic jaundice. Moreover, in these undisputed hemolytic anemias the bone marrow, though it does not actually contain megaloblasts, is extensive and highly cellular, and the dominant erythroid cells are normoblasts or even younger erythroblasts (9, 10). In animal experiments Steele (8) has shown that the immaturity of the erythroid cells of the bone marrow increases with the severity of the anemia produced by blood loss or destruction. Consequently, whether pernicious anemia is an anemia caused by diminished production or by increased destruction of red cells, the stimulus to red cell production will be decreased if the severity of the anemic anoxia is diminished artificially by blood transfusions which do nothing more than raise the hemoglobin concentration in the blood perfusing the bone marrow. Thus, if the development of megaloblasts in the bone marrow in pernicious anemia is partly proportional to the degree of anemic anoxia of the bone marrow, transfusions of blood should have the effect of causing the megaloblasts to diminish in number. The rapid disappearance of the megaloblasts in the bone marrow as a result of their probable conversion to move mature forms as suggested by Schwartz (23) is further evidence against any specific genetic origin of these cells.

The available evidence, however, suggests that both the anoxia of severe anemia and a deficiency of an essential factor for maturation are involved in the production of the classical bone marrow picture in pernicious anemia. According to

to Davidson, Davis, and Innes (15) the administration of liver extract may initiate in 6, and complete in 72, hours the disappearance of the megaloblasts from the bone marrow. This result clearly precedes any significant rise in red cell and hemoglobin values in the peripheral blood. Therefore, it is highly unlikely that the disappearance of the megaloblasts is entirely secondary to a decrease of bone marrow anoxia, as a result of an increased hemoglobin concentration in the blood perfusing the bone marrow. Consequently, the transformation of the megaloblasts cannot be considered to result wholly from the cessation of a hemolytic process, or from increased blood production. Instead, the administration of liver extract must have a direct effect on the rate of maturation of the erythroid cells. It should be understood, however, that this evidence of relative maturation arrest in the formation of megaloblasts does not exclude a hemolytic process as contributory to the anemia, nor does it deny the possibility that liver extract therapy also acts by abolishing a process destructive to reticulocytes, leukocytes, and platelets.

Because deficiency of the active principle of liver extract, as well as the severity of the anemia, can apparently cause immaturity of the erythroid cells of the bone marrow, there is nothing paradoxical in the observation that both liver extract administration and transfusions tend to diminish the megaloblasts in the bone marrow in pernicious anemia. In other types of equally severe anemias, however, immaturity of the erythroid cells does not proceed to the formation of numerous megaloblasts (9, 10). Consequently, it is logical to assume that the anoxia caused by severe anemia is, by itself, insufficient to produce erythroid cells more immature than erythroblasts, unless an additional impediment to the development of their precursors is created by a nutritional deficiency of the active principle of liver extract. Certainly, as was shown by the failure of transfusions to cause the leukocyte and platelet counts to rise, the establishment of normal levels of these elements in the peripheral blood stream also requires the administration of an active principle of liver extract. The close dependency of the red cell, leukocyte, and platelet responses in pernicious anemia upon a common nutritional factor in liver extract is further attested by the similar responses of these

blood elements to pure synthetic *L. casei* factor (3, 4).

SUMMARY AND CONCLUSIONS

1. Rapidly repeated transfusions of blood given to 5 patients with pernicious anemia in relapse, with rises to normal red cell values in 3, failed to produce the striking clinical improvement usually seen following the administration of liver extract, nor did the white cell or platelet counts increase. Thus, as in other nutritional deficiency diseases, much of the clinical improvement in pernicious anemia occurring promptly after administration of the missing nutritional element is presumably a result of specific effects on general cell metabolism, rather than a result of any change in blood values.

2. The previously observed negative effect of transfusions of usual amounts of blood on reticulocytes, leukocytes, and platelets, was shown to extend to larger amounts of blood. Clearly, transfusions do not have hematopoetic effects comparable to those of liver extract administration.

3. Subsequent intramuscular liver extract therapy was followed by rapid clinical improvement, but reticulocyte responses occurred only in those patients with red cell counts below 5 million per cu. mm. A return to normal values of the white cells and platelets was observed at all red cell levels. Thus, liver extract therapy produces a reticulocyte response only when anemic anoxia, the normal stimulus for red cell production, is present; but the responses of white cell and platelets are independent of the degree of anemia.

4. Following the blood transfusions, but prior to the liver extract therapy, the bone marrow megaloblasts characteristic of pernicious anemia disappeared. Others have shown that liver extract therapy also causes these cells to disappear before any significant change occurs in the red cell or hemoglobin levels of the blood. This proves that a state of "maturation arrest," due to a nutritional deficiency, exists. The effect of transfusions suggests that this state is relative rather than absolute, and that it can be affected by an artificial decrease in the degree of bone marrow anoxia.

5. Although relative "maturation arrest" of the bone marrow megaloblasts in pernicious anemia appears to result in part from a deficiency of the active principle of liver extract, this does not exclude increased blood destruction as a contribut-

ing, or even as a dominant, factor in causing the anemia.

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CHEMICAL, CLINICAL AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION: XXXII. THE COAGULATION DEFECT IN HEMOPHILIA. AN *IN VITRO* AND *IN VIVO* COMPARISON OF NORMAL AND HEMOPHILIC WHOLE BLOOD, PLASMA AND DERIVED PLASMA PROTEIN FRACTIONS [1]

By JESSICA H. LEWIS, C. S. DAVIDSON, GEORGE R. MINOT, J. P. SOULIER, H. J. TAGNON, AND F. H. L. TAYLOR

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The clot promoting activity of normal blood or plasma on hemophilic blood has been known for many years. Weil (1) in 1905 first demonstrated that small transfusions of normal blood serum shortened the coagulation time of a hemophilic patient. Minot and Lee in 1912 (2) transfused normal blood into a hemophilic patient and attributed the prompt fall in coagulation time to the added normal platelets. In 1912 Addis (3) showed that normal serum had ability to shorten the hemophiliac's coagulation time both *in vitro* and *in vivo*. In 1924 Feissly (4) demonstrated that small transfusions of normal blood or plasma had an antihemophilic effect, but similar transfusions of hemophilic blood or plasma did not shorten the coagulation time of the recipient hemophiliac.

That the clot promoting ability of normal plasma was independent of the formed elements of the blood was shown by Patek and Stetson (5) and again confirmed by Patek and Taylor (6). The latter authors fractionated Berkefelded plasma, and found this antihemophilic activity to be present in the globulin fraction derived from normal plasma, but markedly reduced or absent in a similar fraction prepared from hemophilic plasma. In the same year Bendien and Van Creveld (7) independently achieved similar results from normal and hemophilic serums. Pavlovsky and Simonetti in 1944 (8), using the technique of Patek and Taylor, found identical hemophilic clot promoting activity in the globulin fractions of normal, thrombocytopenic and fibrinogenopenic plasmas, but in studying the globulin fractions from various hemophilic patients they found the antihemophilic activity variable and usually less marked than normal.

During the past 5 years new methods for fractionation of the plasma proteins have been developed by the Harvard Physical Chemistry Department (9). This paper presents an *in vitro* and *in vivo* comparison of these protein fractions derived from pooled normal and pooled hemophilic plasma. An attempt has been made to identify the defect in the hemophilic blood coagulation mechanism with some of the known components of the blood coagulation reaction. Therefore, the fibrinogen, prothrombin, plasma protease content and the hemophilic clot promoting ability of the 2 plasmas and their derived protein fractions were studied. Also, a comparison was made of the distribution of plasma proteins in normal and hemophilic plasmas as shown by electrophoresis.

METHODS

One thousand ml. of fresh hemophilic plasma fractionated by the Harvard Physical Chemistry Department, using the same methods they had previously applied to

normal plasma. The 5 protein fractions so obtained were tested for fibrinogen, prothrombin, enzyme activity after treatment with chloroform, and antihemophilic activity and were compared with 5 similar fractions from normal pooled plasma. All fractions were dissolved as 2 per cent solutions in 0.85 per cent sodium chloride solution, and the pH adjusted to between 7 and 7.4.

The fibrinogen content was tested by measuring the coagulation time after the addition of 0.1 ml. of thrombin [2] to 0.1 ml. of the protein solution. Prothrombin was determined by a modified Quick test (10) using human brain thromboplastin.

Protease activity was determined after plasma or solutions of the plasma protein fractions were activated by shaking 1 minute with $\frac{1}{10}$ their volume of chloroform. The enzyme activity was estimated by the addition of an equal volume of 1 per cent casein solution (pH 7.5) and measurement of the non-protein nitrogen produced by enzymatic hydrolysis.

Coagulation times were measured by a modification (11) of the method of Lee and White at 37.5° C. *In vitro* estimations of the antihemophilic activity of the various protein fractions were made by the addition of 2 ml. of freshly drawn hemophilic blood to 0.1 ml. of various dilutions of the test material, and subsequent measurement of the coagulation time.

RESULTS

In vitro. Table I shows the components of the protein fractions as prepared by the Physical Chemistry Department of the Harvard Medical School. It will be noted that most of the fibrinogen is in Fraction I, and most of the prothrombin in Fraction II + III. Fraction V is almost pure albumin.

TABLE I

*Distribution of the plasma proteins in normal fractions**

	Albu-min	α-glob-ulin	β-glob-ulin	γ-glob-ulin	Fibrin-ogen	Total protein per liter of plasma
I	0.2	0.2	0.8	0.5	2.6	4.3
II+III	0.7	1.8	6.2	6.0	1.6	16.3
IV	1.0	5.4	3.1	0.2		9.7
V	29.0	0.6				29.6

* Adapted from Cohn, E. J., Oncley, J. L., Strong, L. E., Hughes, W. L., and Armstrong, Jr. S. H., The characterization of the protein fractions of human plasma. J. Clin. Invest., 1944, 23, 417.

Table II shows a comparison of the fibrinogen and the prothrombin content of hemophilic and normal plasma and their derived protein fractions. It is seen that the prothrombin and fibrinogen

[2] Lederle "Clotting Globulin."

content were found to be identical in the 2 plasmas, and similarly distributed in corresponding fractions.

TABLE II

Fibrinogen and prothrombin content of normal and hemophilic plasmas and plasma protein fractions

	Fibrinogen time	Prothrombin Time
Normal: Plasma	3 seconds	20 seconds
Run 183: Fraction I	60 seconds	25 minutes
Fraction II+III	30 minutes	4 minutes
Fraction IV	0	1 minute
Fraction IV–3, 4	0	60 minutes
Fraction V	0	0
Hemophilic: Plasma	3 seconds	20 seconds
Fraction I	60 seconds	2 minutes
Fraction II+III	60 minutes	2 minutes
Fraction IV	0	1 minute
Fraction IV–3, 4	0	0
Fraction V	0	0

The proteolytic activity of both hemophilic and normal plasma and plasma protein fractions, after treatment with chloroform, was found to be very similar.

Figure 1 shows the percentage increase of non-protein nitrogen produced in a casein substrate after treatment for 13 days with chloroform-activated plasma and fractions. The non-protein nitrogen was measured almost daily. There is little activity during the first 12 hours, and the slope of elevation of non-protein nitrogen then rises rather steeply, and at 6 days begins to flatten out. There is no significant difference between the hemophilic and normal curves.

Fractions I, II + III, IV–1 contained most of the proteolytic activity, while the production of non-protein nitrogen following the addition of chloroform-treated Fractions IV–3, 4 and V to a casein substrate is minimal and of the same degree as that produced by an isotonic saline control.

The hemophilic clot promoting activity differed markedly between *normal and hemophilic plasma* and derived fractions, as shown in Tables III and IV. As has been previously shown in this laboratory (12, 13) normal Fractions I and II + III are rich in hemophilic clot promoting substance. Similar fractions obtained from hemophilic plasma showed no such activity. The hemophilic fractions II + III and IV–1 had slight activity, which is probably due to the small amounts of thrombin which have been shown to be present in these materials.
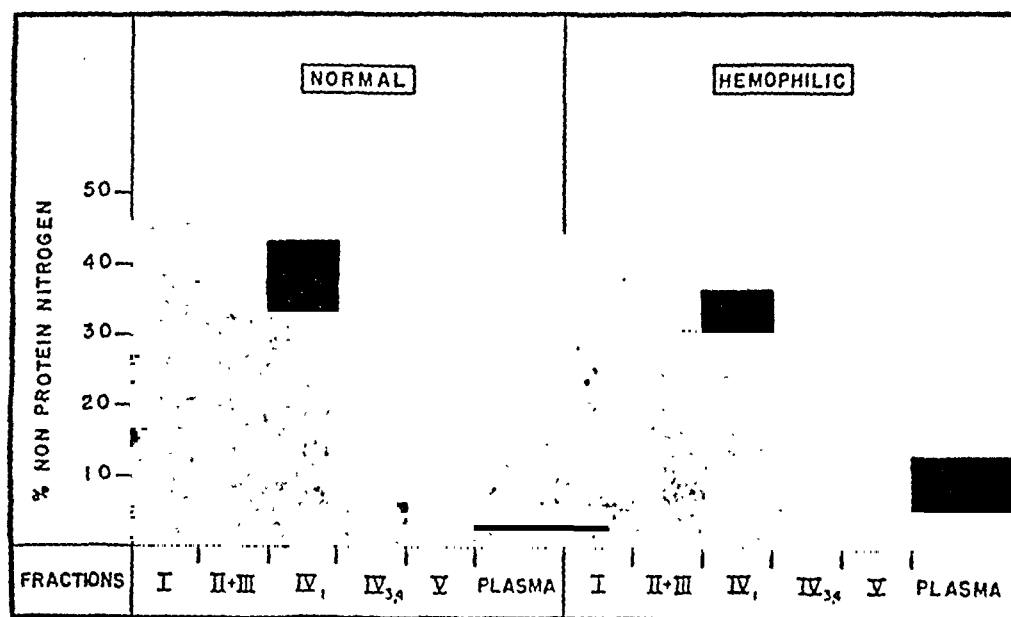
FIG. 1. COMPARISON OF THE PERCENTAGE OF NON-PROTEIN NITROGEN PRODUCED IN A CASEIN SUBSTRATE BY THE PROTEOLYTIC ACTION OF CHLOROFORM-ACTIVATED PLASMA AND PROTEIN FRACTIONS AFTER 13 DAYS

Electrophoretic analysis of 4 hemophilic plasmas were done for us by Dr. S. Howard Armstrong, Jr., in the department of physical chemistry of the Harvard Medical School. In Table V are shown the mean values compared to the normal obtained from pooled plasma. No significant difference was found between the normal and hemophiliac.

In vivo. Transfusions of 100 ml. of compatible blood into hemophilic patients were carried out, using both fresh uncitrated blood injected rapidly intravenously, and citrated blood which had stood at room temperature for 2 hours. Neither of these procedures produced shortening of the recipient hemophiliac's coagulation time.

TABLE III

Antihemophilic activity of normal and hemophilic plasmas

Normal plasma		Hemophilic blood	Coagulation time
dilution	*ml.*	*ml.*	*min.*
0	0.1	2	8
1–10	0.1	2	9½
1–100	0.1	2	17
1–1000	0.1	2	25
Hemophilic plasma			
0	0.1	2	48
1–10	0.1	2	47
1–100	0.1	2	48
1–1000	0.1	2	48
Normal saline			
	0.1	2	42

TABLE IV

Antihemophilic activity of normal and hemophilic fractions

Normal fractions	Anti-hemophilic activity
I	++++
II+III	++
IV–1,	++
IV–3, 4	+−
V	0
Hemophilic fractions	
I	0
II+III	+−
IV–1	+−
IV–3, 4	0
V	0
Saline	0

Figure 2 shows a comparison between the injection of 100 ml. of fresh uncitrated hemophilic blood, followed in 2 hours by the injection of 100 ml. of fresh uncitrated normal blood. There is a marked shortening of the recipient's coagulation time following the normal blood transfusion, but

TABLE V

Comparison of electrophoretic components of hemophilic and normal plasmas

	Albumins	α 1 globulins	α 2 globulins	β globulins	Fibrinogen	γ globulins
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Average 4 hemophilic patients	55.8	5.0	8.5	12.7	6.5	11.5
Average normal pooled human plasma	55.1	5.3	8.7	13.4	6.5	11.0

this did not happen when the hemophilic blood was administered.

Hemophilic plasma was likewise injected into a hemophilic patient without appreciable change in the coagulation time.

Figure 2 shows a comparison of the effect of intravenous transfusion of equal amounts of hemophilic and normal Fraction 1 into the same hemophilic patient. The shortening of the recipient's coagulation time after injection of normal Fraction 1 is in marked contrast to the insignificant change after injection of hemophilic Fraction 1.

It was not unexpected to find the prothrombin and fibrinogen content of the hemophilic protein fractions to be similar to the corresponding normal fractions. This is a confirmation of the generally accepted fact that the coagulation defect in hemophilia is not due to a deficiency of fibrinogen or prothrombin.

Thrombin is not normally present in the circulating blood, but appears during the blood coagulation reaction. In hemophilia, the conversion of prothrombin to thrombin is impaired. This is probably not due to the presence of an anticoagulant, as hemophilic blood or plasma added to normal blood does not prolong the coagulation time of the normal blood. It appears therefore, that the delayed formation of thrombin in hemophilia is due to a deficiency of one or more of the precursors of thrombin. In view of the fact that the coagulation defect in hemophilia is corrected by the addition both *in vitro* and *in vivo* of small amounts of fibrinogen, prothrombin and platelet-free plasma (14), and that the prothrombin content of hemophilic plasma is normal, the conclusion seems inescapable that there is a precursor of thrombin other than prothrombin which is deficient in hemophilic plasma.

This substance has been termed "globulin substance" by Taylor (6), "plasma thromboplastin" by Howell (15) and "plasma thromboplastic enzyme" by Ferguson (16). These 3 authors agree that the active substance is derived from plasma,
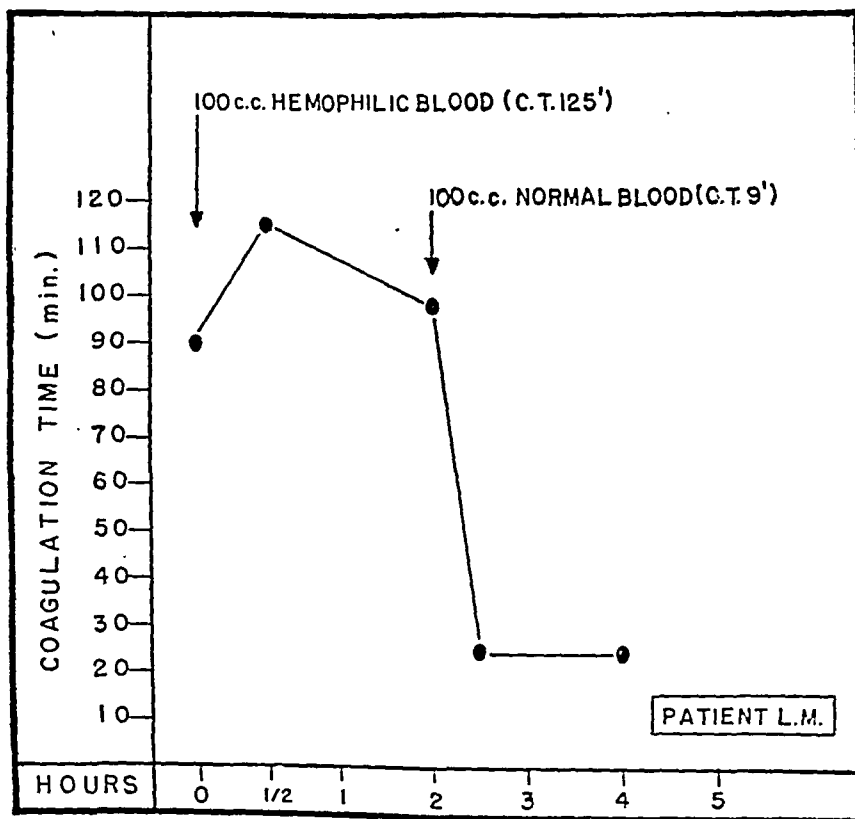
FIG. 2. A COMPARISON BETWEEN THE INJECTION INTO A HEMOPHILIC PATIENT OF HEMOPHILIC AND NORMAL BLOOD
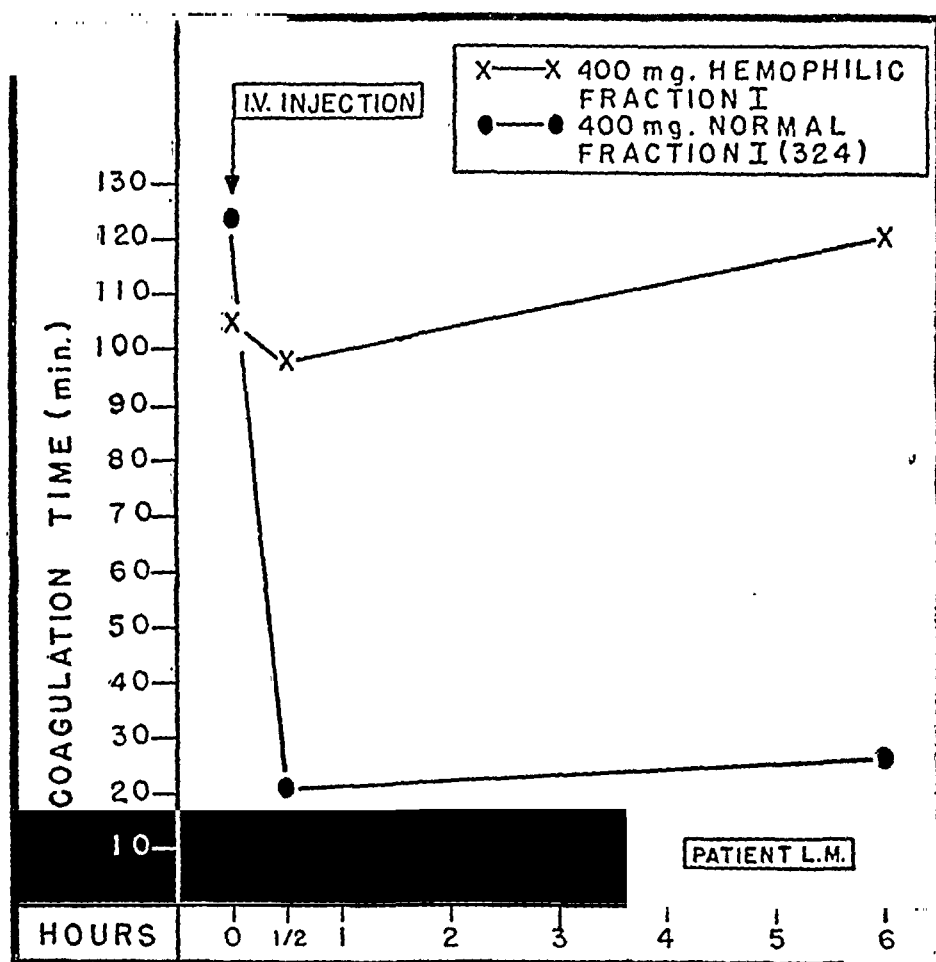
FIG. 3. A COMPARISON BETWEEN INTRAVENOUS INJECTIONS OF EQUAL AMOUNTS OF
HEMOPHILIC AND NORMAL FRACTION I INTO THE SAME HEMOPHILIC PATIENT

but little is known as to its chemical nature. The term "globulin substance" used by Taylor implies that the active material is associated with the globulin fraction of the proteins of plasma, but is not meant to convey the idea that it is a globulin or even a protein. Ferguson has advanced indirect evidence indicating that the proteolytic enzyme and the antihemophilic factor of plasma are identical, but direct proof of this is lacking. Tocantins (17) suggests an antithromboplastic substance present in hemophilic plasma which inhibits the normal thromboplastic action and thus produces defective blood coagulation.

Tagnon, Davidson and Taylor (18) have compared the fibrinolytic activity of chloroform-treated normal and hemophilic plasmas, and observed that the fibrinolytic activity was reduced in hemophilic plasma. The results presented in this paper are in contrast to these in that they show little difference in proteolytic activity between normal and hemophilic pooled plasma, or their corresponding protein fractions. This discrepancy may be due to the difference in technique used in either investigation. The protease activity of the normal and hemophilic fractions seems to be identical but while the protease activity is parallele by the presence of antihemophilic activity in the normal plasma protein fractions, there is no such relationship in the hemophilic fraction.

A plasma factor deficient in hemophilia could not be identified with fibrinogen, prothrombin, or the proteolytic enzyme. The deficiency of this factor in hemophilic plasma does not alter the Tiselius diagram. Further attempts are being made to isolate this factor by subfractionation of the active globulin fractions with which it is associated.

CONCLUSIONS

1. Normal and hemophilic plasmas are similar in detectable protein composition and distribution.
2. The fibrinogen and prothrombin content are identical.

3. The proteolytic activity of the 2 plasmas, or their corresponding protein fractions after treatment with chloroform, is identical.

4. Normal plasma and normal Fractions, I, II + III and IV-1 contain a substance capable of shortening hemophilic coagulation time. This substance is absent in hemophilic plasma and its corresponding fractions.

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CHEMICAL, CLINICAL AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION. XXXIII. THE COAGULATION DEFECT IN HEMOPHILIA: THE EFFECT *IN VITRO* AND *IN VIVO* ON THE COAGULATION TIME IN HEMOPHILIA OF A PROTHROMBIN AND FIBRINOGEN–FREE NORMAL PLASMA AND ITS DERIVED PROTEIN FRACTIONS [1,2,3]

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Patek and Taylor (1) in 1937 demonstrated that the activity of normal plasma in shortening the coagulation time of hemophilic blood could be found almost quantitatively in a derived globulin fraction. In 1939, Lozner, Kark and Taylor (2) showed that Berkefelded normal plasma free from fibrinogen and prothrombin had hemophilic clot-promoting power similar to that of untreated normal plasma. In unpublished investigations (3), they found that a globulin fraction derived from this fibrinogen and prothrombin-free plasma also had antihemophilic activity. Pavlovsky and Simonetti (4) in 1944, using the technique of Patek and Taylor, demonstrated that globulin substance prepared from the plasma of a patient with congenital fibrinogenopenia had normal antihemophilic activity.

Investigation in this laboratory of plasma protein fractions prepared by the Harvard Physical Chemistry Department showed marked *in vitro* (5) antihemophilic activity to be present in Fractions I and II + III. Fraction I studied *in vivo* (6) likewise showed marked power to shorten the coagulation time of hemophilic patient's blood.

This investigation was undertaken to study the antihemophilic activity of the various plasma protein fractions prepared by the Harvard Physical Chemistry Department from plasma free of fibrinogen and prothrombin.

METHODS

Fifteen hundred ml. of plasma obtained from normal human blood within 4 hours after venesection was heated to 56° C. for 2½ minutes. The precipitate was removed by centrifugation, and the supernatant plasma tested for fibrinogen and prothrombin. Prothrombin time was determined by a modification (7) of the Quick technique (8) using a human brain thromboplastin. Fibrinogen was measured by clot formation after addition of 0.1 ml. thrombin [4] to 0.1 ml. plasma.

The heated plasma was rapidly frozen and preserved at − 20° to − 40° C. until fractionation. The plasma protein fractions were dissolved in sufficient 0.85 per cent sodium chloride solution to make a 2 per cent solution by weight, and the pH adjusted at 7 to 7.4 before use. Samples of the original plasma and heated plasma were kept at − 20°, and tested for antihemophilic activity at the same time as the plasma protein fractions. The plasmas and plasma protein fractions were diluted by 0.85 per cent sodium chloride solution to make serial dilutions of 1/5, 1/50, 1/500, 1/5,000 and 1/50,000.

Antihemophilic activity was measured by 2 methods. In the first method, the time of coagulation was measured after 0.1 ml. of 0.25 per cent calcium chloride solution was added to a mixture containing 0.1 ml. of hemophilic plasma, 0.7 ml. of 0.85 per cent sodium chloride solution, and 0.1 ml. of the various dilutions of the 2 per cent solutions of the protein fractions. In the second method, whole blood was used instead of the diluted plasma. A modification of the Lee and White coagulation method (9) was employed, in which the coagulation of the hemophilic whole blood was measured after the addition of 2 ml. of the blood to 0.1 ml. of the various dilutions of the plasma protein fractions. All coagulation reactions were carried out at 37.5° C. in a constant temperature water bath.

[4] Lederle clotting globulin.

RESULTS

Table I shows the prothrombin and fibrinogen content of the plasma before and after heating to 56° C. for 2½ minutes. After heating, there is no clottable fibrinogen, and only a trace of prothrombin in the plasma. Table II shows the distribution of protein in the fractions of normal and heat defibrinogenated plasma. Part of the albumin, as well as the fibrinogen and prothrombin, had been coagulated by the heat processing. No fibrinogen or thrombin was found in any of these plasma protein fractions, and in Fractions I, II + III and IV only traces of prothrombin were detected.

Tables III and IV show the hemophilic clot promoting activity of the original plasma, heated plasma and plasma protein fractions as measured by hemophilic plasma recalcification, and whole blood coagulation times. These data indicate that some of the antihemophilic activity of whole plasma is lost by the heating to 56° C. for 2½ minutes, although the prothrombin and fibrinogen free plasma thus obtained still has marked hemophilic clot promoting powers. The fractions derived from this material show marked activity in Fractions I and II + III, and are very similar to fractions obtained from normal plasma (5).

Sterile preparations of normal Fraction I were heated unopened to 56° C. for 4 minutes, and the

TABLE I

The effect of 56° C for 2½ minutes on the prothrombin and fibrinogen of normal citrated plasma

	"Prothrombin time"*	"Fibrinogen time"†
Normal plasma	13 seconds	4 seconds
Heated plasma	‡ 10 minutes	no clot in 24 hrs.

* Human brain thromboplastin.
† Lederle clotting globulin used as thrombin.
‡ 0.1 ml. standard fibrinogen solution added.

TABLE II

Distribution of protein among fractions of normal and heat defibrinogenated plasma

Fraction	Normal protein	Heat defibrinogenated protein
	grams per 1000 ml.	*grams per 1000 ml.*
I	4.3	2
II+III	16.3	14
IV–1	9.7	3
IV–4		3
V (albumin)	29.6	15
Total	59.9	37.0

TABLE III

Recalcification time (in minutes) after addition of various dilutions of whole plasma, plasma freed from fibrinogen and prothrombin, and derived plasma protein fractions, to diluted hemophilic plasma

Dilution	0	$\frac{1}{5}$	$\frac{1}{50}$	$\frac{1}{500}$	$\frac{1}{5000}$
Saline (control)	26				
Whole plasma	2	2½	4½	8	14
Heated plasma	3½	5	9	14	21
Fraction I	4	5	6	9½	15½
Fraction II+III	4	6	10	16	23
Fraction IV–1	17	20	25	27	27
Fraction IV–4	21	21½	29	26	26
Fraction V	27	26	26	26	26

TABLE IV

Coagulation time (in minutes) of hemophilic whole blood after addition of various dilutions of whole plasma, plasma freed from fibrinogen and prothrombin and its derived plasma protein fractions

Dilution	0	$\frac{1}{5}$	$\frac{1}{50}$	$\frac{1}{500}$	$\frac{1}{5000}$	$\frac{1}{50,000}$
Saline (control)	123					
Whole plasma	9	9½	19	19	21	56
Heated plasma	12	12½	19	48	102	97
Fraction I	10	16½	65	74	105	112
Fraction II+III	9	17	51	102	112	112
Fraction IV–1	74	102	112	112	128	123
Fraction IV–4	84	102	123	123	123	123
Fraction V	92	93	112	117	123	117

supernatant fluid collected sterily and injected intravenously into a hemophilic patient. Figure 1 shows a comparison of the response of one hemophilic patient to intravenous injection of 0.8 gram of Fraction I and the supernatant obtained after heating the same amount of the same Fraction I. The protein contents of these solutions vary markedly, the total protein of the former being 450 mgm. injected, and of the latter 190 mgm. injected; but the antihemophilic activities are very similar, demonstrating that the fibrinogen-free Fraction I has not lost much activity in the heating process.

DISCUSSION

These investigations again emphasize that the substance present in normal blood which will shorten the coagulation time of hemophilic blood is independent of fibrinogen and prothrombin content of the blood, as well as the formed elements. This substance is asociated with the globulin fraction of the plasma proteins, and essentially with Fractions I and II + III as prepared by the Harvard Physical Chemistry Department. This
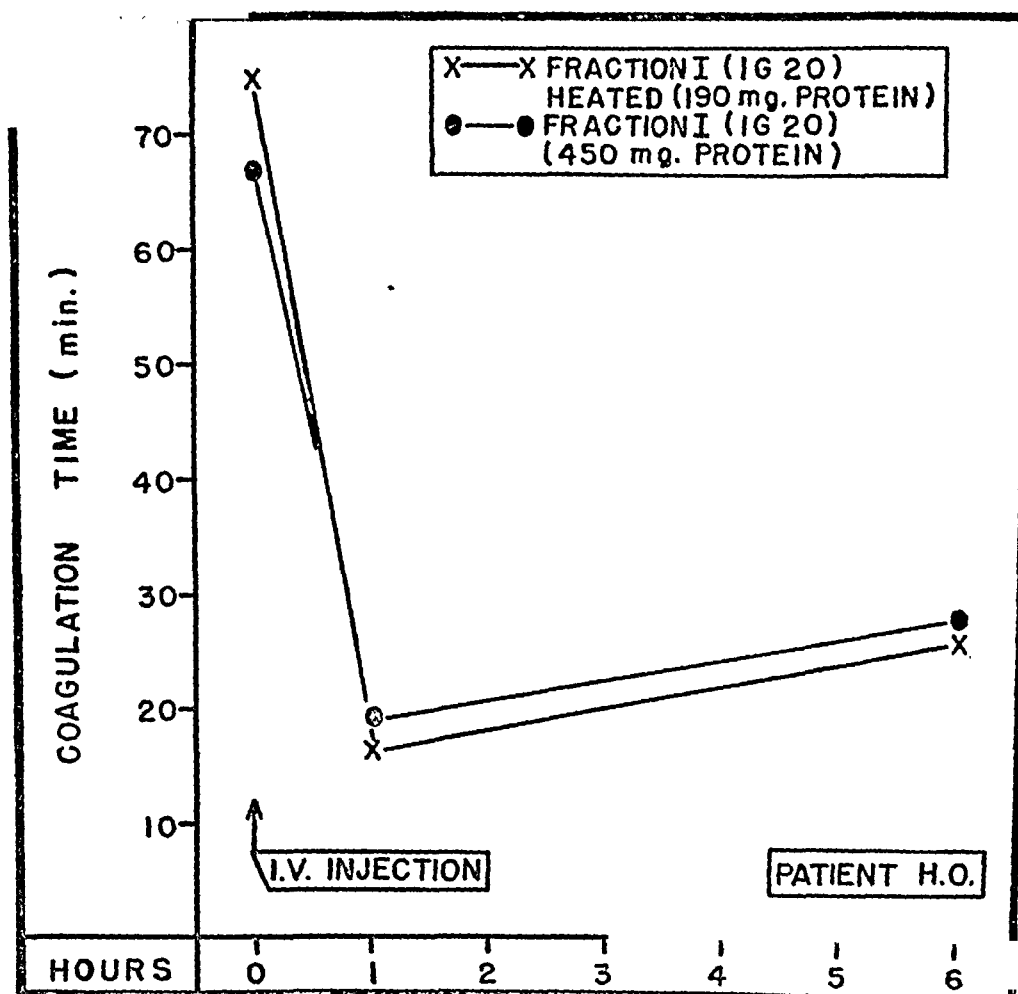
FIG. 1. A COMPARISON OF THE ANTIHEMOPHILIC ACTIVITY OF NORMAL FRACTION I
AND HEAT DEFIBRINOGENATED FRACTION I IN THE SAME PATIENT

globulin substance is more thermostabile at 56° C. than the other known protein components of the blood coagulation reaction.

Fraction I prepared from this heated plasma contains no clottable fibrinogen, but is fairly insoluble in both water and various dilutions of saline which may be due to the presence of denatured fibrinogen. This material was not given to patients intravenously. Instead, Fraction I prepared from normal plasma and then heated to coagulate the fibrinogen was used. The supernatant contains no clottable fibrinogen and a low protein content, but is as active in reducing the coagulation time of a hemophilic patient as the same Fraction I before heating. This suggests that it may be possible to prepare an antihemophilic substance in a concentrated form.

CONCLUSIONS

1. Fifteen hundred ml. of normal plasma heated at 56° C. for 2½ minutes contained no clottable

fibrinogen and only a trace of prothrombin.

2. This heated plasma and protein fractions prepared from it contain antihemophilic activity similar to normal plasma and plasma protein fractions.

3. A solution of Fraction I from which the fibrinogen has been removed by heat coagulation is as active as the original Fraction I in reducing the coagulation time of a hemophilic patient when injected intravenously.

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STUDIES ON HYPERVENTILATION. II. ELECTROCARDIOGRAPHIC CHANGES IN NORMAL MAN DURING VOLUNTARY HYPERVENTILATION [1,2]

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Any normal human being will present certain cardiac symptoms, besides the well-known tetany signs, during voluntary hyperventilation, which removes carbon dioxide. Thus a feeling of precordial oppression, in some cases increasing to proper precordial pains with eradiation to the left arm, and often accompanied by an unaccountable sensation of fear, will generally develop after 1 or 2 minutes of hyperventilation. Continued hyperventilation for another 2 or 3 minutes will, in many cases, bring about a pronounced feeling of air hunger.

The above signs and symptoms are suggestive of the presence of a transitory coronary insufficiency. It therefore seems reasonable to find out whether signs of coronary insufficiency can be demonstrated electrocardiographically during voluntary hyperventilation.

A number of reports have been published on electrocardiographic examinations during hyperventilation. Kronenberger and Ruffin state that they have observed flattening of the T waves in one or more leads in 8 cases during attacks of hyperventilation tetany, whereas electrocardiograms registered between these attacks presented perfectly normal conditions. These writers hold that the changes occurring during the hyperventilation are caused by an increased vagal tonus. McCance (2) has observed similar changes during attacks of hyperventilation in 2 patients, but he is unable to give an explanation of the occurrence of these changes. Lami (3), who has experimented with 25 normal subjects, has also found a flattening of the T waves together with a decrease in the heights of the R waves in one or more leads during voluntary hyperventilation. This writer is of the opinion that the changes are due to a change of position of the heart brought about by a local

tetany in the diaphragm. Barker and collaborators (4) have observed the same changes in 4 normal subjects during voluntary hyperventilation, and have, besides, managed to bring about such changes by administration of large amounts of sodium bicarbonate. These writers are likewise unable to give an explanation to the occurrence of the changes. Barach and Steiner (5) have regarded the problem from the opposite side, in so far as they have tried to find out, whether it should be possible to change a pathological electrocardiogram by letting patients suffering from coronary sclerosis breathe in air containing carbon dioxide. The result of their experiments was that in 20 out of 26 cases the T waves became slightly higher in one or more leads during respiration of air containing carbon dioxide; and they suppose that the changes are due to the coronary vessels being dilated during the acidosis which is brought about by the carbon dioxide.

The report here presented is the outcome of 40 experiments carried out on 4 normal persons between the ages of 15 and 42. In some of the experiments, only the 3 usual extremital leads have been registered, while in others also the precordial lead 4R has been applied. All deflections were registered synchronously, except in the case of the first person. The rate was calculated on the basis of the time elapsing between 8 successive R waves. The electrocardiograph was for all registrations adjusted in such a manner that a difference of tension of 1 millivolt brought about a deflection of 10 mm. The measurement of the heights of the individual waves was carried out according to Kaj. H. Larsen's method (6), in which the straight line tangent to the lower border of the curve on the segment between the end of the P wave and the beginning of the initial complex is used as the iso-electric level. This line does not always run a horizontal course, but may be either slightly ascending or descending. In such cases the straight line tangent to the curve at the beginning of the

initial complex is used as the iso-electric level. The latter must be the straight line tangent to the lower border of the curve between the end of the T wave, or sometimes the U wave, and the beginning of the P wave, as the electrocardiographic curve between the end of the P wave and the beginning of the initial complex will sometimes run a few tenths of a millimeter below the T–P segment. The stated heights of the individual waves have, like the indicated times, been calculated as the average of the respective values of 5 successive complexes.

METHODS

The experiments were carried out in the morning and were commenced after the subjects, who were fasting, had lain in rest for 1 hour in the laboratory. After this period of rest, 2 electrocardiograms were registered at intervals of 5 minutes in order to make sure that the electrocardiogram presented no spontaneous changes of any note. Next the hyperventilation was started in atmospheric air. During the hyperventilation the subject tried to increase both depth and rate of respiration as much as possible. Three to 5 electrocardiograms were registered within this part of the experiment, which lasted from 3 to 8 minutes. In some of the experiments the spontaneous decline of the electrocardiographic changes was studied next by registration of from 5 to 10 electrocardiograms within up to half an hour. In the remaining experiments the hyperventilation was continued, with pure oxygen being given in some cases, and a mixture of 5 per cent carbon dioxide, 20 per cent oxygen, and 75 per cent nitrogen in others. Finally some experiments were made in which 2 mgm. nitroglycerin were given immediately before the commencement of the hyperventilation.

RESULTS

When double experiments were carried out, the changes always proved to be fairly similar in both sets of electrocardiograms; and great accordance was likewise demonstrated between the changes observed for the different subjects.

Some of the electrocardiograms are rather unsatisfactory from a technical point of view, because the registrations had to be made during strong hyperventilation, and in a few cases, at a point of time at which the subject presented a manifest tetany.

Electrocardiographic changes during voluntary hyperventilation. Voluntary hyperventilation brings about pronounced and characteristic changes in the electrocardiogram, as will be demonstrated.

Figure 1 illustrates the action of the heart of a girl aged 17. The first set, which was registered before the hyperventilation was commenced, shows quite normal conditions. The second set, which was registered after 5 minutes of hyperventilation
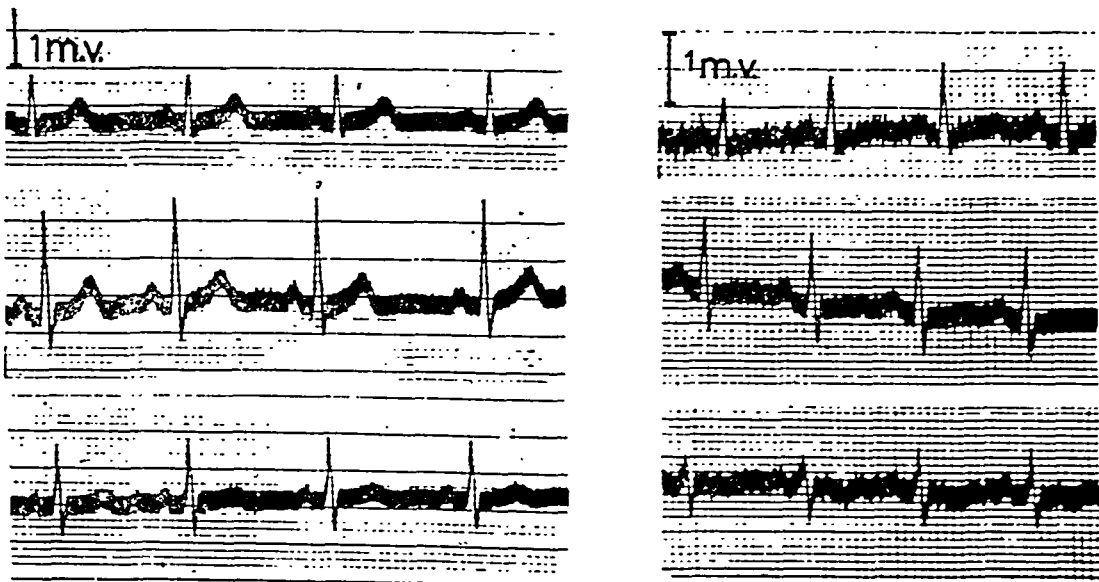
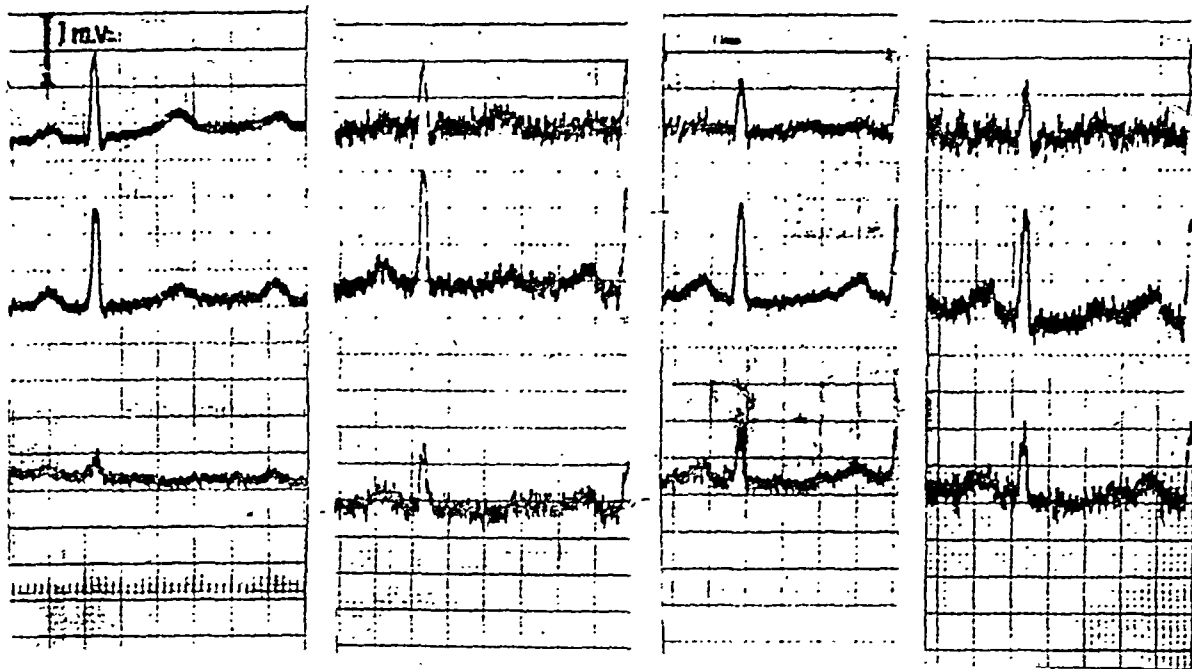FIG. 1. ELECTROCARDIOGRAMS FROM SUBJECT No. 1

The first EKG was registered during normal respiration; the second EKG, after 5 minutes of hyperventilation in atmospheric air.

A

in atmospheric air, shows the following changes: The rate has increased from 85 to 110. T_1 has decreased from 2½ mm. to 1 mm.; T_2, from 3½ to 0 mm.; and T_3, from $+1$ to -1 mm. At the same time R_{2-3} have become somewhat lower.

The second person experimented on was a girl aged 15 (Figure 2). The first set was registered before the beginning of the experiment; the second set, after hyperventilation for 2 minutes; and the third set, after 5 minutes of hyperventilation. The hyperventilation is here seen to have changed, particularly the S–T segment and the T waves in the second and third leads. The S–T segment has become depressed 1 to 1½ mm., and at the same time T_{2-3} have become iso-electric or nega-tive. The sizes of the individual changes appear from Table I.

The next experiment to be mentioned was made on a woman aged 42. Figure 3 shows first a set of normal electrocardiograms, which were registered immediately before the beginning of the experiment. The next 5 electrocardiograms were registered after hyperventilation for 2, 3, 4, 6, and 7 minutes respectively. The precordial electrode failed to act at the registration of the 2 last electrocardiograms. Very massive changes, particularly of the S–T segment and the T waves, are seen here in all 4 leads. The sizes of the individual changes appear from Table II.

The fourth person experimented on was a wo-

TABLE I

The electrocardiographic changes presented by subject no. 2 during voluntary hyperventilation
Time indicated in seconds. Height of waves in mm.

Point of time	Rate	P-Q	Q-T	P_1	P_2	P_3	R_1	R_2	R_3	S-T$_1$	S-T$_2$	S-T$_3$	T_1	T_2	T_3
Before hyperventilation	62	0.16	0.34	1	2	1	5	17	14	0	0	0	1	3½	2½
a. 2 min. of hyperventilation	120	0.16	0.26	1	2¼	1¼	3	18	16	0	−1	−1¼	0	−1	−1
a. 5 min. of hyperventilation	120	0.14	0.26	1	2¼	1¼	4	16	13	0	−1½	−1½	0	−2	−1½
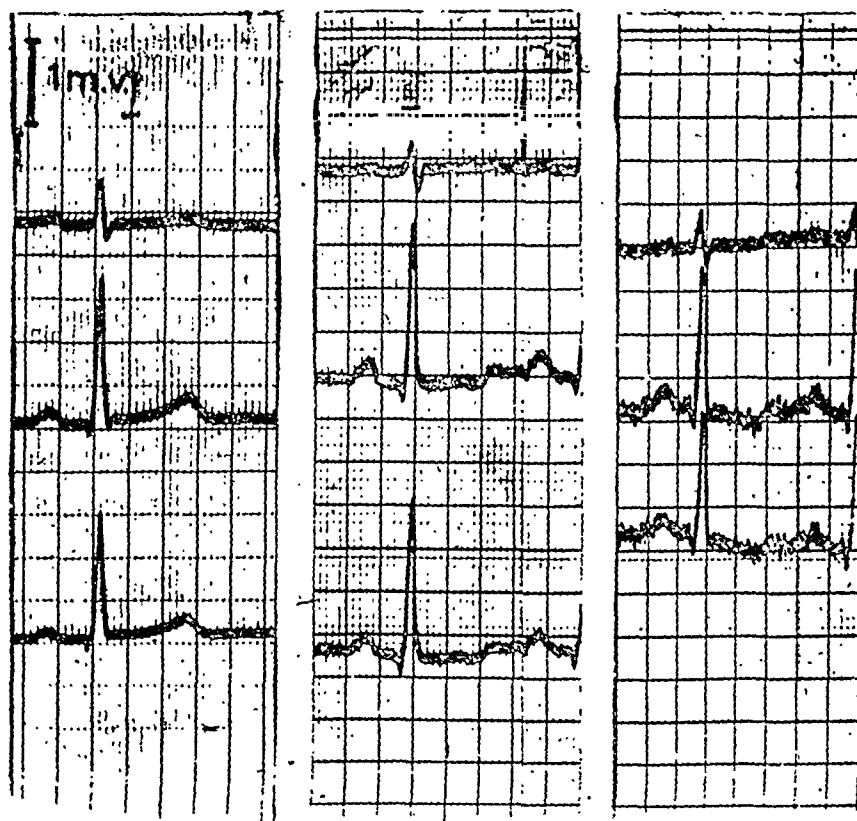
FIG. 2. ELECTROCARDIOGRAMS FROM SUBJECT No. 2

The first EKG was registered during normal respiration; the second and third sets, after hyperventilation in atmospheric air for 2 and 5 minutes respectively.
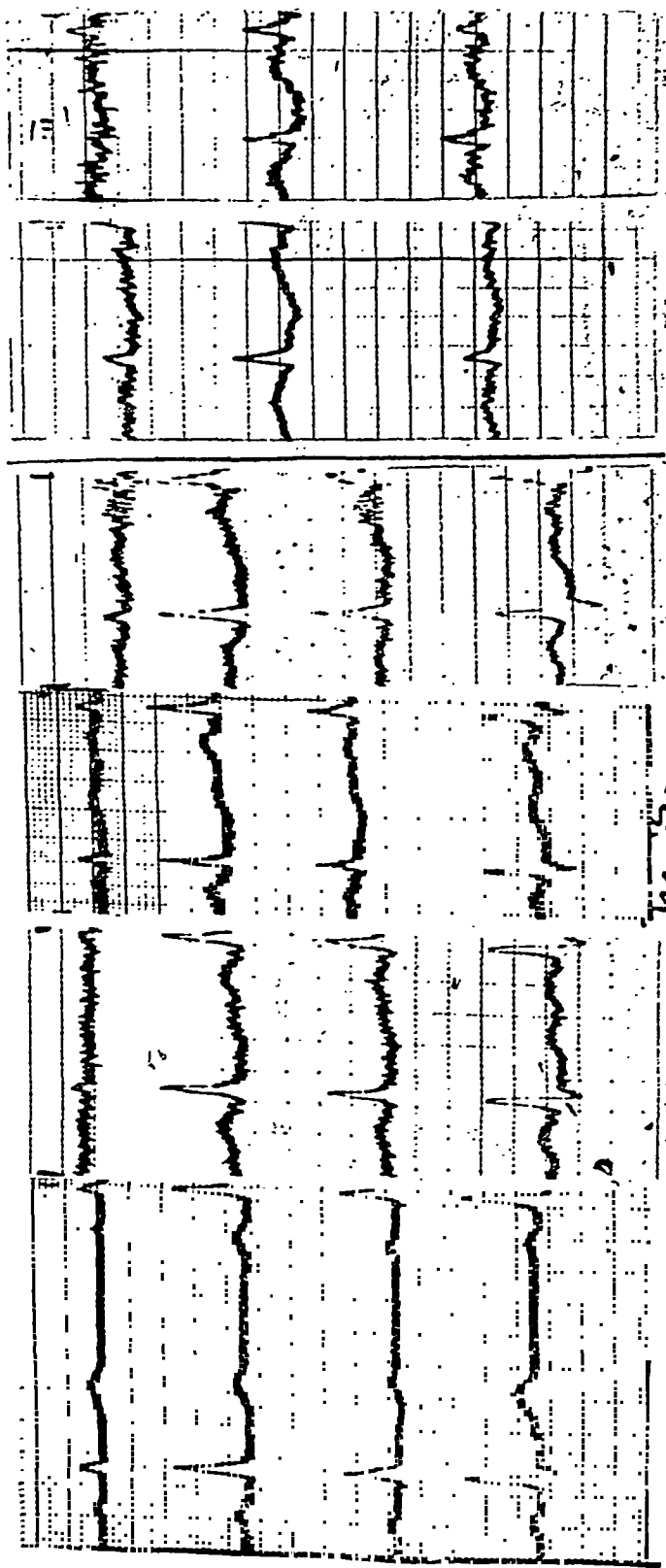
FIG. 3. ELECTROCARDIOGRAMS FROM SUBJECT No. 3

The first EKG was registered during normal respiration; the second, third, fourth, fifth, and sixth sets were registered after hyperventilation in atmospheric air for 2, 3, 4, 6, and 7 minutes respectively.

TABLE II

The electrocardiographic changes presented by subject no. 3 during voluntary hyperventilation
Time indicated in seconds. Height of waves in mm.

Point of time	Rate	P-Q	Q-T	P₁	P₂	P₃	P₄R	R₁	R₂	R₃	R₄R	S-T₁	S-T₂	S-T₃	S-T₄R	T₁	T₂	T₃	T₄R
Before hyperventilation	72	0.32	0.37	1	2	1	1½	3	12	8	11	0	0	0	0	1½	2	*	4
a. 2 min. of hyperventilation	125	0.14	0.28	½	1½	1	1½	2	12	8	9	0	−1½	−1	−1	0	0	0	1½
a. 3 min. of hyperventilation	120	0.14	0.30	1	2	1	1	3	10	6	8	0	−1½	−1	−1½	1	0	0	1½
a. 4 min. of hyperventilation	150	0.14	0.28	1	1½	1	1½	2	12	10	8	0	−2	−1	−2	1	0	0	1
a. 6 min. of hyperventilation	130	0.14	0.28	1	1½	1		3	8	4		+1	−2	−1		−1	−2	−1	
a. 7 min. of hyperventilation	166	0.14	0.22	1	1½	1		3	7	4		?	−1	−1		?	−2	−1	

* Diphasic.

man aged 30 (Figure 4). The first set of electrocardiograms was registered before the hyperventilation was commenced; and the following 3, after hyperventilation for 3, 4, and 6 minutes respectively. This experiment likewise revealed pronounced changes of S–T segment and T waves in all 3 leads. The sizes of the individual changes appear from Table III.

The electrocardiographic examinations during voluntary hyperventilation in atmospheric air have shown that the following changes occur:

(1) sinus tachycardia

(2) a slight reduction of the time of conduction

(3) a shortening of the electric systole duration, so that the latter falls within the range of the normal values indicated by Kaj H. Larsen (7)

(4) no indisputable change of the heights of the P waves

(5) uncharacteristic changes of the heights of the R waves

(6) depression of the S–T segment of at least 3 mm. or more in 2 or more leads

(7) iso-electric, diphasic, or inverted T waves in 2 or more leads.

These electrocardiographic changes are highly indicative of a coronary insufficiency, since the criteria required by Levy, Williams, Bruenn, and Carr (8) are represented to the full, viz.,

(1) A total depression of the S–T segment in the 3 extremital leads and 4F of no less than 3 mm., or

(2) depression of S–T₁ of at least 1 mm. together with total or partial inversion of T₁, or

(3) a negative T₄F with no other changes, or

(4) iso-electric or diphasic T₄F as well as depression of S–T₄F of at least 1 mm.

The spontaneous decrease in the electrocardiographic changes after the discontinuation of the hyperventilation was studied for all 4 experimental subjects. These examinations showed that the electrocardiogram becomes fairly normal after 5 minutes and is perfectly normal again after 10 minutes.

The electrocardiographic changes during continued hyperventilation in pure oxygen were likewise studied for all 4 women. In one case only did the electrocardiographic changes disappear after 6 minutes of hyperventilation in pure oxygen. In the other cases there was observed no decrease in the electrocardiographic changes.

The electrocardiographic changes during continued hyperventilation in a mixture of 5 per cent carbon dioxide, 20 per cent oxygen, and 75 per cent nitrogen. All the changes proved to disappear in the course of between 30 and 60 seconds in the cases of all 4 women.

The electrocardiographic changes during voluntary hyperventilation immediately after adminis-

TABLE III

The electrocardiographic changes presented by subject no. 4 during voluntary hyperventilation
Time indicated in seconds. Height of waves in mm.

Point of time	Rate	P-Q	Q-T	P₁	P₂	P₃	R₁	R₂	R₃	S-T₁	S-T₂	S-T₃	T₁	T₂	T₃
Before hyperventilation	90	0.14	0.32	1¼	2½	1	12	14	2	0	0	0	3½	2½	−1
a. 3 min. of hyperventilation	107	0.14	0.30	1	2½	1	9	14	6	−1	−2	−1	2	1	−1
a. 4 min. of hyperventilation	143	0.13	0.26	1	2½	1½	6	13	7	−1	−1	0	1	0	−1
a. 6 min. of hyperventilation	131	0.12	0.30	1	2½	1½	7	15	8	−1	−2½	−2	1	0	0

FIG. 4. ELECTROCARDIOGRAMS FROM SUBJECT No. 4

The first EKG was registered during normal respiration; the second, third, and fourth sets were registered after hyperventilation for 3, 4 and 6 minutes respectively.

tration of 2 mgm. nitroglycerin. Double experiments with 2 of the women showed that administration of 2 mgm. nitroglycerin has no influence on the occurrence of the electrocardiographic changes.

DISCUSSION

The experiments mentioned in this paper show that distinct electrocardiographic changes indicative of coronary insufficiency will occur in normal man during voluntary hyperventilation in atmospheric air. But there is seen no prolongation of the electric systole duration; nor should we expect to find such a prolongation after Schultzer and Lebel's (9) demonstration of the fact that the concentration of calcium ions does not decrease during attacks of hyperventilation tetany.

It has been maintained (3) that the transitory changes in the electrocardiogram may be due to a change of position of the heart caused by a local tetany of the diaphragm. It is, however, not very likely that a change of position would be able to bring about the changes mentioned here, which apply particularly to S–T segment and T waves; nor is it possible by registration of electrocardiograms at the stage of deepest inspiration to demonstrate

changes that on any point resemble those described here. It is therefore to be supposed that the changes are due to a reduction in the supply of oxygen to the myocardium. This must be taken as a sign that the precordial sensations appearing during hyperventilation in atmospheric air are of myocardial origin.

A transitory reduction in the supply of oxygen to the myocardium must have been caused by changes in the factors conditioning the liberation of oxygen during the flowing of the blood through the capillaries, either on account of changed circumstances of dissociation for the oxyhemoglobin, or on account of changed conditions of flowing.

Bohr, Hasselbalch, and Krogh (10) have proved that the dissociation of the oxyhemoglobin is impeded by a reduction of the carbon dioxide tension in the blood. This phenomenon has been studied further by Barcroft and collaborators (11), who plotted the curves illustrated in Figure 5.

Figure 5 shows the dissociation curves of the oxyhemoglobin at different carbon dioxide tensions. The figures below the abscissa axis indicate the oxygen tension in mm. Hg, while the ordinate indicates the percentage of oxyhemoglobin;

and the figures above the individual curves indicate the respective carbon dioxide tensions. Under normal conditions, the oxygen tension is 100 mm., and the carbon dioxide is 40 mm. in arterial blood, approximately. In venous blood, the corresponding tensions are 40 and 45 mm. respectively. The oxyhemoglobin will thus, under normal conditions, give off about 5 volumes per cent of oxygen during the flow of the blood through the capillaries. During hyperventilation, carbon dioxide tension can easily be reduced to 20 mm., while at the same time the oxygen tension remains unchanged. There occurs no rise in the carbon dioxide tension in the venous blood, because there is found an excess of fixed cations to bind the produced carbon dioxide, and the fall of the oxygen tension does not exceed the normal. It follows from this that the liberation of oxygen is impeded rather considerably under such conditions. It appears from Figure 5 that, at a carbon dioxide tension of about 20 mm. Hg, the blood gives off only 2.5 volumes per cent of oxygen during its flow through the capillaries. At respiration in atmospheric air the physically bound amount of oxygen in the blood is so small (0.20 volume per cent), that it is of no significance. But if the per-

son experimented on breathes in pure oxygen, the physically bound oxygen will increase to a rather considerable amount. Under such conditions, the oxygen tension in the arterial blood rises to nearly 700 mm. Hg, whereas, on account of the great fall of tension in the tissues, it is normal (40 mm.) in the venous blood. At the above tensions the arterial blood contains approximately 1.75 volumes per cent, and the venous blood, approximately 0.10 volume per cent of physically bound oxygen, which gives an extra oxygen liberation in the capillaries of about 1.65 volumes per cent. This figure added to the previously mentioned 2.5 volumes per cent, which is liberated from the oxyhemoglobin, gives 4.15 volumes per cent, a figure so high that this amount should be able to make the signs of myocardial hypoxemia subside, if the latter had been brought about exclusively by the phenomena mentioned above. Our experiments with continued respiration in pure oxygen show, however, that the signs of hypoxemia could be removed in one case only, and even in that case not till after 6 minutes had elapsed. This must be regarded as a proof that other factors must play a greater part than the changed dissociation of oxyhemoglobin.

In all the experiments of continued respiration in a mixture of 5 per cent carbon dioxide, 20 per cent oxygen, and 75 per cent nitrogen, the electrocardiographic signs of myocardial hypoxemia disappeared within less than a minute. This seems indicative that the transitory coronary insufficiency is due to the acapnia and the alkalosis, and that the changed hemodynamic conditions caused by the hyperventilation itself play no part.

The tachycardia caused by the hyperventilation can have no influence on the occurrence of the electrocardiographic changes, since no such changes are seen during hyperventilation in air rich in carbon dioxide, although a pronounced tachycardia is present also in these experiments.

The arterial blood pressure undergoes no changes during the hyperventilation (12), so accordingly the demonstrated myocardial hypoxemia must have been caused either by changes in the state of constriction of the coronary vessels, or by an increased intramyocardial pressure resulting in inhibited flowing of blood, or finally by a combination of these factors.

It has been shown, through numerous animal experiments (13 to 18), that a reduced carbon
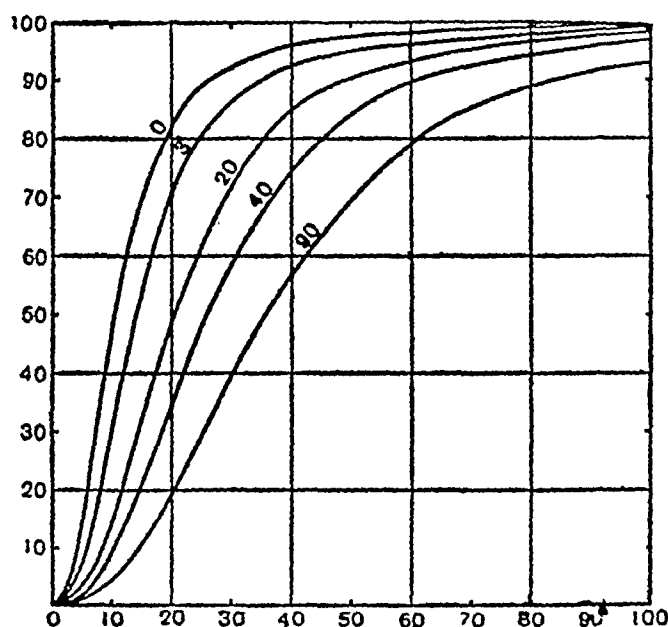
FIG. 5. DISSOCIATION CURVES OF OXYHEMOGLOBIN AT DIFFERENT CARBON DIOXIDE TENSIONS

The abscissa indicates the oxygen tension in mm. Hg; and the ordinate, the percentage of oxyhemoglobin; the figures above the individual curves indicate the respective carbon dioxide tensions.

dioxide tension, or an otherwise produced shift of the reaction of the passing fluid towards the basic side, brings about a pronounced constriction of the coronary vessels. Thus a shift of the pH from 7.35 to 7.70 will reduce the coronary flow by approximately 50 per cent. Yandell Henderson (19) has proved, in convincing experiments on dogs, that the volume of the heart decreases remarkably during hyperventilation in such a way that the diastolic volume is reduced first and then the systolic volume, after which the heart finally stops in the systole. As administration of large doses of nitroglycerin immediately before the beginning of the hyperventilation has no influence on the occurrence of the electrocardiographic changes, it seems reasonable to conclude that constrictions of the coronary vessels can be of no essential importance for the appearance of the myocardial hypoxemia during hyperventilation. Accordingly, there is reason to presume that the transitory myocardial hypoxemia developed during hyperventilation is conditioned in the first instance on a reduced flowing of blood through the coronary vessels on account of a compression of the latter, due to an increased intramyocardial tension.

The facts demonstrated through these experiments are of rather considerable practical importance in various cases. Thus, for instance, there have been demonstrated light, transitory, electrocardiographic changes in the febrile periods of nearly all febrile, infectious diseases. These changes have been taken as signs of a transitory, toxic affection of the myocardium, an explanation which is probably correct in some cases; but on the other hand it should be borne in mind that febrile patients present a rather pronounced hyperventilation, even more pronounced than what corresponds to the increase in the metabolic rate. Considering that even a slight hyperventilation can bring about small changes in the electrocardiogram, we cannot leave out of account the possibility that a contingent hyperventilation may have a share in the above changes.

Finally, also, the electrocardiographic, functional tests and the conclusions drawn from them should be discussed in brief. In electrocardiographic examinations after muscular work, or during respiration of a mixture of air poor in oxygen, it is generally presumed that an electrocardiographically demonstrated coronary insufficiency,

according to Levy's criteria, is an unquestionable sign of a possible latent coronary sclerosis. This can, however, hardly be sanctioned as a general rule available to all cases, for it is a well-known fact that untrained individuals hyperventilate during muscular work, so that accordingly there should be a possibility that electrocardiographic changes, if present, might have been brought about by acapnia. This fact must play a rather considerable part in the cases in which the differential diagnosis stands between coronary affection and the effort syndrome, as the author has previously pointed out (20) that patients suffering from the effort syndrome hyperventilate so much during muscular work that there occurs a pronounced vasoconstriction of the big extremital arteries. Distinct electrocardiographic changes have in some cases been observed (21) in apparently healthy young people at the ordinary hypoxemia tests, where the subject is made to breathe in a mixture of air consisting of 10 per cent oxygen and 90 per cent nitrogen. This can easily be explained as having its cause in the involuntary hyperventilation, which occurs in all individuals who breathe in air that is poor in oxygen.

The facts just reported argue, in my opinion, in favor of using the hypoxemia test, and not the working electrocardiogram, for the diagnosis of latent coronary affections, and of using for the hypoxemia test a mixture of air which contains 2 or 3 per cent carbon dioxide in order to counteract the acapnia brought about by the hyperventilation.

SUMMARY

1. Voluntary hyperventilation, which removes carbon dioxide, brings about distinct electrocardiographic signs of myocardial hypoxemia in normal human beings. These signs disappear again in the course of about 10 minutes after the discontinuation of the hyperventilation.

2. Continued hyperventilation in pure oxygen brings about no definite decrease in the electrocardiographic changes. This means that apparently the changes are not due exclusively to the dissociation of the oxyhemoglobin, having been impeded by the reduced carbon dioxide tension.

3. Continued hyperventilation in a mixture of 5 per cent carbon dioxide, 20 per cent oxygen, and 75 per cent nitrogen makes the electrocardio-

graphic changes disappear within less than a minute. Consequently, it is to be supposed that the changes are due to the acapnia and the alkalosis. Numerous animal experiments have been carried out, on the basis of which it seems justifiable to conclude that the myocardial hypoxemia is brought about either by a constriction of the coronary vessels, or by an increased intramyocardial tension, or by a combination of these 2 factors.

4. Administration of large doses of nitroglycerin immediately before the commencement of the experiment does not prevent the occurrence of the electrocardiographic changes. This goes to show that such changes cannot be caused by a coronary constriction alone, but must be due chiefly to an increase in the intramyocardial tension.

5. Finally, it is pointed out that the facts demonstrated through these experiments may be of rather considerable practical importance at the estimation of electrocardiograms registered after bodily exertion or during respiration of air deficient in oxygen, since an involuntary hyperventilation brought about by these procedures may result in electrocardiographic changes in normal human beings.

CONCLUSIONS

Voluntary hyperventilation, which removes carbon dioxide, brings about distinct electrocardiographic signs of myocardial hypoxemia in normal human beings.

The myocardial hypoxemia demonstrated electrocardiographically is probably due to the increased intramyocardial tension caused by the acapnia and the alkalosis. At the estimation of electrocardiograms registered after bodily exertion or during respiration of oxygen-deficient or carbon dioxide-free air, one should bear in mind the fact that these procedures will in many cases bring about an involuntary hyperventilation, which may cause electrocardiographic changes to occur.

Addendum. On account of the difficult conditions of communication during the war, I did not learn about William Paul Thompson's investigations from 1943 (22) till after the conclusion of the work here presented. Thompson has demonstrated electrocardiographic changes of a kind similar to those described here in patients with the hyperventilation syndrome, and in normal persons during hyperventilation. He is of the opinion

that such changes are due to the alkalosis. detailed description is given, however, of mechanism of the occurrence of these changes.

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MEASUREMENTS RELATED TO PAIN IN NEUROCIRCULATORY ASTHENIA, ANXIETY NEUROSIS, OR EFFORT SYNDROME: LEVELS OF HEAT STIMULUS PERCEIVED AS PAINFUL AND PRODUCING WINCE AND WITHDRAWAL REACTIONS[1]

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Multiple complaints of discomfort such as aches, pains and distress are a common finding in patients with neurocirculatory asthenia (N.C.A.). Among the symptoms listed in our patients with this condition are chest pain 92 per cent, headaches 66 per cent, "indigestion" 64 per cent, marked self-observation including observation of disagreeable symptoms 64 per cent. In addition, discomfort seems to affect the behavior and lead to great disability in these patients. In attempting to put through a program of training, it was found that patients would not submit to the various exercises proposed by the trainer (1). The patients stated that exercise made them too uncomfortable and led to aches and pains.

It was felt, therefore, that investigation of the patients' level of awareness of painful stimuli, *i.e.* perception, and the patients' reaction to painful stimuli, should be studied. For the purpose of studying the threshold at which patients perceived a standard stimulus as painful, and the level at which the patient reacted (winced or pulled away), we employed the Hardy-Wolff radiation apparatus (2).

METHOD

On grounds of history alone, 2 groups of patients were distinguished. Those who had a life long course, or who could never do hard work or take part in athletics, were called chronic N.C.A.; those who gave convincing evidence of good health, good work or athletic ability and nervous stability previous to onset of the disorder were called acute N.C.A. Measurements were made in 94 patients, members of the armed forces, of whom 63 were cases of chronic N.C.A., and 31 cases of acute N.C.A.

The control subjects were 44 healthy individuals, 36 of whom were in military service, and 8 medical students. Patients and controls were men, and were roughly comparable as regards age range, economic, social and ethnic groups. Additional control subjects were 17 convalescent soldiers recovering from infected bone injuries sustained in combat or while in military service.

The stimulus consisted of varying intensities of heat supplied by the radiation apparaus (3). The source of heat was a 1,000 watt Mazda lamp with the light focused onto the midline of the forehead by 2 plano-convex lenses through an aperture 2.5 cm.² in area. To standardize the absorption of light, the forehead was first blackened with India ink. Each exposure was kept constant at 3 seconds by means of a shutter operated by a telechron motor, and the intensity was varied uniformly by a wire rheostat. The amount of stimulus used was calibrated periodically by a radiometer, and expressed in absolute end point values of gram cal. per sec. and cm.² of skin surface.*

Each subject was tested on 2 separate occasions, but never on the same day. With few exceptions, each test consisted of from 10 to 14 exposures to the stimulus, with approximately a 2-minute interval between exposures. Before the start of the first test, no comments were made or instructions given, other than to tell the subject to keep his eyes closed during each stimulus. After each exposure the subject was asked these non-committal questions: (*1*) "What did you feel?" (*2*) "How would you describe what you felt when the stimulus was most intense?" (*3*) "Was the stimulus as intense, less or more intense than the previous one?" (*4*) "Was the stimulus like anything you have ever felt before?" (A card with 7 numbered circles varying from the diameter of a silver dollar, 4 cm., to a pin head, 1 mm., was held before the subject.) He was asked which circle corresponded in area to the size of the place on his forehead where the stimulus seemed most intense. The purpose of these questions was to have the patient describe what he felt when the stimulus was applied, avoiding suggesting the answer.

We also wished to learn whether N.C.A. subjects described their feelings at the painful level in the same or in a different manner from the control subjects.

The second test was administered in a slightly different

* This is commonly expressed as gm. cal./sec./cm.²

manner from the first. The subject was given the following description of the stimulus: "At the beginning, the exposures feel like a warm glow. As they become more intense, the sensation feels smaller and hot or burny. Eventually, the most intense part of the stimulus changes from a burn to a sharp piercing quality about the size of a pencil point." He was also asked to keep his forehead at the aperture until told to move it away.

The lowest level of stimulus at which the subject said he felt a sharp jabbing or piercing sensation, *i.e.*, the *endpoint for perception*, was calculated as the mean of the consistent values plus the inconsistent values. A consistent value was one at which the subject felt the sharp, jabbing or piercing sensation, but it must be one of a series on a given day in which there were no failures at perceiving the endpoint sensation. An inconsistent value was one at which the subject felt the endpoint sensation at least once but also failed to do so on one or more subsequent exposures at that level. Any values which were higher than the consistent ones were excluded. The *threshold of motor reaction* was taken to be the lowest level at which the subject winced, as evidenced by a beginning contraction of the muscles at the outer canthus of the eye. This value was calculated in the same manner as that for the subjective endpoint of pain, that is, by taking the mean of the consistent value plus the inconsistent value.

From the first exposure where a warm glow was felt, to levels where the sharp jabbing endpoint was felt, and where wincing occurred, the stimulus was varied from .030 to .040 gram cal. per sec. and cm.² each time. In order to obtain as closely as possible the endpoint levels, the stimulus was altered as little as .005 or .010 gram cal. per sec. and cm.² each time. At the completion of each test, notations were made regarding factors such as nervousness, restlessness, taking of medication or alcohol. If the subjects had taken either analgesic medication or alcohol, no test was performed on that day. When both tests were finished, the word "pain" was mentioned by the examiner for the first time. The subject was asked to define "pain," and to state whether he thought the sharp, jabbing end point seemed like a "pain" sensation.

Also, a certain number of subjects were asked to recall if they winced, and to state why they winced.

The results of these tests were then correlated with other clinical and laboratory findings to determine their possible interrelationship.

Most of the tests were made by the same examiner. To rule out any variation which might be due to the tester, another physician performed the test on 18 of the control subjects and 15 of the patients.

RESULTS

(A) Lowest level at which stimulus is perceived as painful, and at which patient winces or pulls away

Figure 1 presents the data from our second test for the level at which the stimulus was perceived

as painful by each patient. The levels at which control subjects and patients with neurocirculatory asthenia perceived the stimulus as painful are almost identical in the 4 groups.

However, when the means of the levels at which stimuli produce wince reaction are compared, patients with chronic N.C.A. react at the lowest level, acute N.C.A. at the next level, convalescents wince at a higher level and the healthy control subjects wince at the highest levels of all (Figure 2). The differences between the chronic N.C.A. and the control groups are significant statistically (Table II).

In addition to the wince reaction, many of the patients pulled their heads away from the apparatus. This response occurred (Table III) frequently in patients and almost not at all in healthy and convalescent controls. At levels lower than .351 gram cal. per sec. per cm.², 3 per cent of healthy control subjects pulled away, 7 per cent convalescents, 32 per cent acute N.C.A. patients, and 49 per cent chronic N.C.A. patients pulled away. The differences at this stimulus level between the N.C.A. patients and the control subject, both healthy and convalescent, are highly sig-

TABLE I

Summary of data on levels at which stimulus is perceived as painful (perception) and at which wincing occurs (reaction)

Perception—Test II

Subjects	No.	Range	†Mean	*S.D.	**C. of V.	***S.E.
Controls, healthy	44	.241 to .356	.2872	.024	8.2	.0036
Controls, convalescent	17	.225 to .320	.2834	.022	7.8	.0053
N.C.A., acutes	30	.245 to .348	.2918	.027	9.2	.0049
N.C.A., chronic	62	.225 to .460	.2866	.027	9.5	.0034

Reaction—Test II

Subjects	No.	Range	†Mean	*S.D.	**C. of V.	***S.E.
Controls, healthy	44	.249 to .447	.3636	.054	15.0	.0082
Controls, convalescent	17	.235 to .410	.3574	.059	17.0	.0143
N.C.A., acute	31	.234 to .416	.3261	.058	18.0	.0104
N.C.A., chronic	63	.225 to .460	.3140	.070	22.0	.0090

*S.D. = Standard Deviation.
**C. of V. = Coefficient of Variation.
***S.E. = Standard Error.
†Mean in Gm. cal./sec. and cm.²
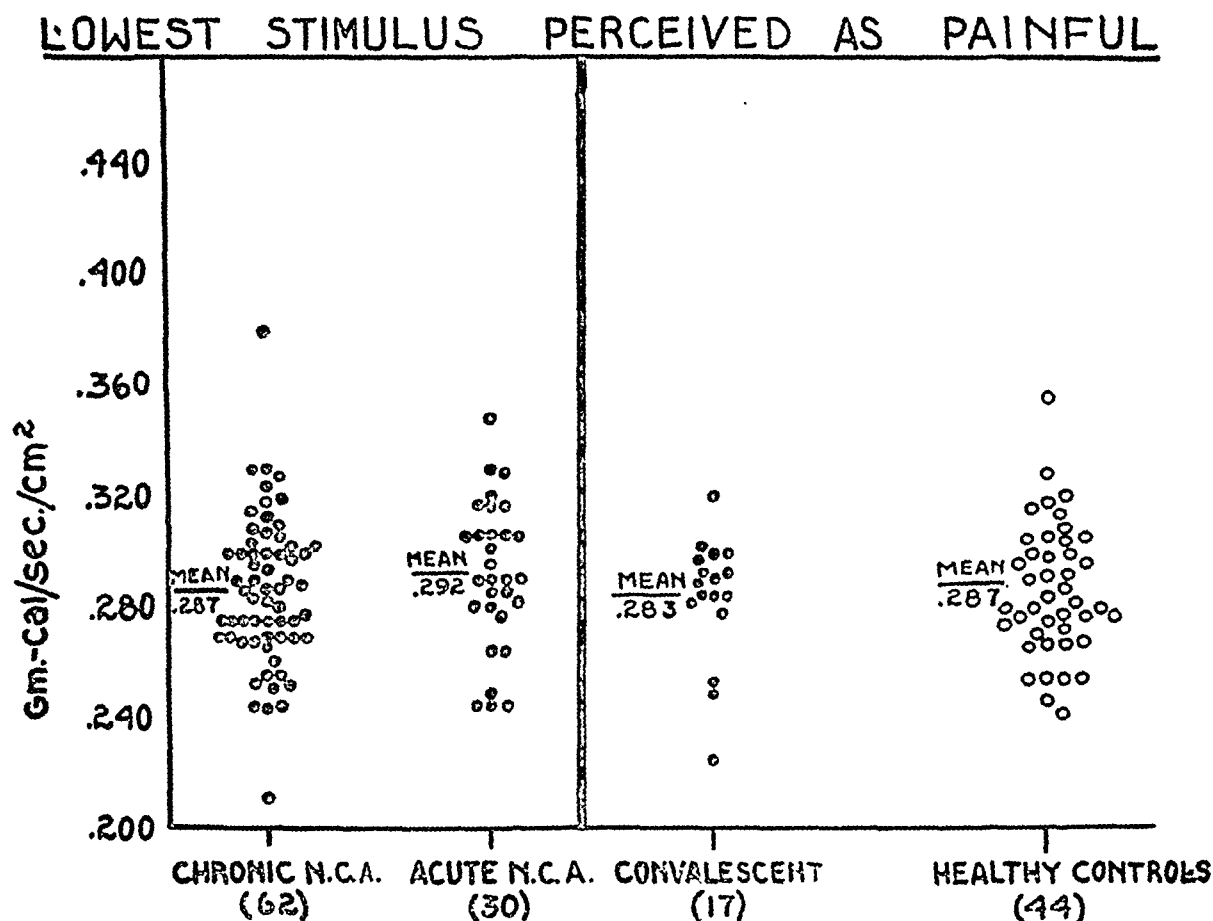
FIG. 1. THE LOWEST LEVEL OF HEAT STIMULUS PERCEIVED AS PAINFUL IN EACH INDIVIDUAL SUBJECT IS PRESENTED ABOVE AS A DOT OR CIRCLE

The levels are indicated on the ordinate in gram cal. and sec. per cm.². The number and type of subjects are indicated along the abscissa. This figure shows that the mean of the observation is the same for all groups. The slight differences are not significant; the distributions are similar for the 4 groups. Above data are charted from values obtained in test II in each case.

TABLE II

Significance of the difference between the means of the various groups

	Chronics vs. healthy	Acutes vs. healthy	Chronics vs. convalesc.	Convalesc. vs. healthy	Acutes vs. convalesc.
Test II—*Perception* Difference between means					
Standard error	.0046	.0058	.0062	.0061	.0070
Significance ratio	.13	.80	.52	.63	1.19
Reaction Difference between means					
Standard error	.0120	.0132	.0168	.0164	.0176
Significance ratio	4.12	2.85	2.58	.38	1.77
Difference (test II–test I): *Reaction*					
Standard error	.0071	.0081	.0127	.0135	.0133
Significance ratio	.95	.62	.26	.74	.38
Individual spread *Perception*					
Test I difference.....	.0027				
Standard error	.0004				
Significance ratio	.66				
Test II difference	.0021				
Standard error	3.89				
Significance ratio	.54				

nificant, with a significance ratio of 5.50. Table III also illustrates that at the lowest stimulus level patients and control subjects do not differ particularly, but, as the stimulus is increased, the differences between patients and control subjects in withdrawal becomes more marked. At the 3 highest stimulus levels, a greater proportion of chronic patients pull away from the apparatus than do the

TABLE III

Percentage incidence of pulling head away from apparatus at various heat levels (Controls vs. N.C.A.)

At level, gram cal. per sec. and cm.², of *less than*	Gram cal. per sec. per cm.²			
	.276	.301	.326	.351
Healthy (28)	0	0	0	3
Convalescent (15)	0	0	0	7
Acute N.C.A. (23)	8	21	30	32
Chronic N.C.A. (50)	5	27	38	49

acute patients. The difference is not, however, statistically significant.

We conclude from the above observations that the level at which the stimulus was perceived as painful is not significantly different from the patient group to the control group. The wince level, however, is lower for the patients than for the controls. The pullaway response occurs more frequently and at a lower stimulus level in the patients than it does in the control subjects.

(B) Consistency of levels of perception and reaction in a given individual during a test

In an individual case there is a spread between the level at which the patient *first* perceives the stimulus as painful, and the lowest level at which he *consistently* perceives the level as painful. This is called the spread in the level of perception. The same phenomenon is also true of reaction, namely, a spread between the level at which the patient first winces, and the lowest level at which the pa-

tient winces consistently. The mean levels for test I and II are given in Table IV. The spread of the perception levels does not vary particularly from test I to test II. The means for the spread in the level of reaction are slightly lower in test II than in test I.

(C) Comparison of perception and reaction levels, test I to test II

The patients and controls were compared as to the variability in results from test I to test II, and also as to the degree of consistency of the endpoints both in perception and reaction during a given test. As shown in Table IV, the results from the second test showed but slight differences from those of the first test.

During the first test, the patients were given no instructions as to the nature of the sensation, but were instructed to look for the sharp, jabbing endpoint during the second test. The results of the 2 tests are almost identical as seen from Table
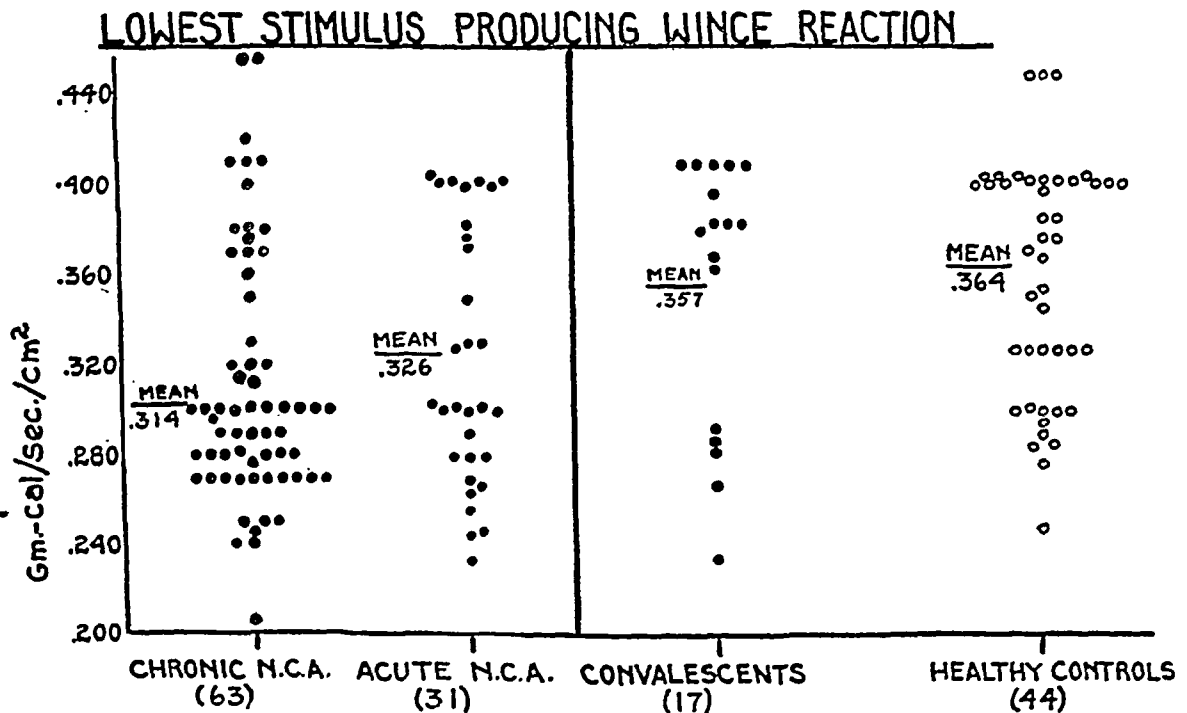
FIG. 2. THE LOWEST HEAT STIMULUS PRODUCING WINCE REACTION IS REPRESENTED BY A DOT OR CIRCLE FOR EACH SUBJECT

The levels are indicated on the ordinate in gram cal. per sec. and cm.^{2} The number and type of subjects are indicated along the abscissa. The mean values are significantly lower in both groups of N.C.A. than in healthy control subjects. The group in the chronic N.C.A. differs significantly from the convalescent control group, and from the acute N.C.A. group as well. The above data were obtained from test II in each case.

TABLE IV

Test II vs. test I. Comparison of mean results of perception, reaction and consistency

	No.	I	II	Difference (test II-test I)	Significance ratio
Mean perception					
Healthy	31	.2845	.2821	−.0024	.70
Convalescent	12	.2785	.2775	−.0010	.26
Acute	14	.3011	.2942	−.0069	1.43
Chronic	36	.2840	.2861	+.0021	.61
Wince level					
Healthy	29	.3343	.3404	+.0061	.55
Convalescent	15	.3342	.3503	+.0161	1.33
Acute	28	.3074	.3185	+.0111	2.00
Chronic	57	.2899	.3027	+.0128	3.26
Spread in level—perception					
Healthy	31	.0126	.0129	+.0003	
Convalescent	12	.0095	.0058	−.0037	
Acute	14	.0096	.0124	+.0028	
Chronic	36	.0153	.0150	−.0003	
Spread in level—reaction					
Healthy	44	.0200	.0100	−.0100	
Convalescent	17	.0200	.0200	.0000	
Acute	31	.0300	.0240	−.0060	
Chronic	63	.0230	.0120	−.0110	

IV. None of the differences between these means was great. From this we conclude that the level at which the stimulus is perceived as painful is fairly consistent from test to test in the same individual, and that doing the procedure with either technique yields the same results. Also there was no difference in results depending on the tester.

In contrast, the wince level tends to be slightly higher in the second test than in the first one, that is, the patient stands more stimulus before he winces when tested a second time. This is true of all 4 groups, but only in the N.C.A. patients are the differences from test I to test II statistically significant (Table IV).

(D) *Was the perception endpoint really perceived as painful?*

In answer to the question whether the sharp, jabbing endpoint was regarded as painful, the subjects were asked (1) to define pain, (2) whether the sharp jabbing endpoint was "a pain." Both

the patients and controls regarded pain as some form of hurting sensation. The sharp jabbing endpoint was regarded as a painful stimulus in all but 3 of 79 subjects. The reason given for calling the pain perception endpoint "painful" was almost always that it had a "hurting" quality.

(E) *Description of pain endpoint in N.C.A. as compared with controls*

At the level at which the stimulus was perceived as painful, the patients described the pain in an identical manner as the control subjects, that is, as a sharp jabbing, sharp piercing, sharp pricking of a needle.

(F) *Subject's explanation for wincing*

The patient was asked why he winced. Among the common explanations given were "it hurt," "it was a painful sensation," "it helped me withstand the pain." Some also stated that they didn't know or were not aware that they had winced.

(G) *Relationship of wince level to other data*

The data in patients were examined to discover if there was any relationship between wince levels and other findings such as the work index, blood lactate levels associated with work, ventilation index, familial incidence of this condition, number of symptoms in the present illness and in the past medical history, and the type of chief complaint. With the exception of chief complaint, there was no obvious relation between the wince levels and any of the above factors, although no planned intensive study of all possibilities was made.

The wince level of 22 patients whose chief complaint was "nervousness" was .301 gram cal. per sec. and cm.², in contrast to .329 gram cal. per sec. and cm.² in 56 patients with chief complaints other than "nervousness." The difference between the levels is statistically significant (significance ratio 2.08, odds against this being a chance occurrence, are 25 to 1), demonstrating that there is a lower wince level in patients with chief complaints of "nervousness" as compared to those in the other chief complaints such as chest pain, weakness, breathlessness, headache, and dizziness.

(H) Is there a relation in individual cases between perception and reaction levels?

When individual data for perception and wince levels were correlated in the 4 groups, it was found that in each group the reaction level depended on the level of perception. The higher the perception level the higher was the level of reaction

The values for the correlation coefficients are: chronic N.C.A., .64; acute N.C.A., .29; convalescent controls, .50; healthy controls, .53, all but the second being significant. Inasmuch as the mean perception level in all 4 groups was the same, the low wince levels in the patient groups could not be explained by a low perception level.

SUMMARY OF RESULTS

(A) 1. Patients with N.C.A. perceive the heat stimulus as painful at the same level as do the control subjects.

2. Patients with N.C.A. wince at a lower level of stimulus than do the controls.

3. This is also true for the levels at which they pull away from the apparatus, a higher proportion of patients withdrawing at each level and at lower levels as compared with the controls.

4. Patients with chronic N.C.A. react (wince, pull away) at lower levels than do patients with acute N.C.A.

(B) The degree of consistency of the levels at which each stimulus is perceived as painful, and at which wincing occurs, was no different in patients and controls during a given test.

(C) 1. Perception levels do not differ significantly from test I to test II.

2. Wince levels tend to be slightly higher in test II; this is significantly so in both groups of N.C.A. patients.

3. Similar results were obtained in perception level, whether subject is instructed to look for jabbing sensation or is given no instruction and volunteers a description of this endpoint.

4. There is no change in consistency in pain or wince level from test I to test II.

(D) The subjects report the stimulus as a jabbing, piercing, hurting sensation and agree that it is painful.

(E) Patients and control subjects use the same type and intensity of wording in describing the heat stimulus at the pain perception endpoint.

(F) Patients explained that they winced because "it hurt" or to "withstand the pain."

(G) There was a significantly lower wince level in patients with chief complaint of nervousness than in patients with the chief complaints other than nervousness, such as chest pain, headache, weakness, breathlessness, or dizziness. There was no other correlation of wince level with other findings from history, or from other tests.

(H) There is positive correlation in individual cases between levels of perception and reaction (wince).

DISCUSSION

This test measures the amount of heat stimulus which is perceived as painful on the mid-line of the forehead. The fact that this is the same for patients and controls does not imply that the same would hold for other locations, for visceral pain, or for other unpleasant stimuli. No support is offered by this finding for the idea that these patients complain more of discomfort because they feel pain with less stimulus. The patients' description makes it certain that it is a pain that is experienced and not some other sensation, as far as one can be certain of sensation.

The reaction level offered an estimation of the amount of stimulus at which motor reaction occurred.

The exact neurologic pathways of these responses are not known, nor is the entire significance of them understood. The fact that the patients with "nervousness" as a chief complaint show the lowest wince levels, suggests that the factor of "nervousness" may be an important factor in the low wince levels in N.C.A. In other studies, low wince level has been shown to be related to age and ethnic group of subject (3), but these factors are not important in this study, as comparable controls were used.

The finding of a low wince level in N.C.A. corresponds with a similar finding in a previous study (4) in various types of neurosis. This provides a further point of correspondence between neurocirculatory asthenia and patients commonly placed in the neurosis category (5). In this paper neurocirculatory asthenia, anxiety neurosis, and effort syndrome are considered as synonyms describing one syndrome (6).

The patients' reaction at a lower stimulus level

corresponds to the patients' poor performance in holding an uncomfortable grip dynamometer, poor performance in breath holding, and in general to patients' statement of inability to tolerate discomfort (1).

It is of further interest that the values check from test I to test II and during each test, despite the subjective nature of part of the procedure. The checks are as good in the patients as in controls, suggesting that patients of this type may be consistent witnesses of subjective data.

This test might be used as an aid in the diagnosis of neurocirculatory asthenia, effort syndrome, and anxiety neurosis. A wince level above .400 gram cal. per sec. and cm.² makes a diagnosis of neurocirculatory asthenia improbable, as fewer than 10 per cent of patients are above .400. A wince level below .290 gram cal. per sec. and cm.² is corroboration for the diagnosis of neurocirculatory asthenia, it being improbable that such a subject is normal, as only 10 per cent of healthy controls fall below this level.

CONCLUSIONS

1. The level at which patients with neurocirculatory asthenia, effort syndrome or anxiety neurosis *perceive* the heat stimulus as *painful* is the same as that for control subjects.

2. In contrast, the level at which the N.C.A. patient *reacts* to the heat stimulus (winces, pulls away) is lower than that for the control subjects.

3. This difference is most marked in chronic N.C.A.; less in acute N.C.A.

4. N.C.A. patients with nervousness as a chief complaint wince at a lower wince level than do N.C.A. patients with other chief complaints.

5. The reaction of N.C.A. patients at a low stimulus level offers a quantitative correlate of the clinical impression of the patients' inability to stand discomfort.

6. This test may be of use in establishing the diagnosis of neurocirculatory asthenia, effort syndrome, or anxiety neurosis.

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RENAL REABSORPTION OF CHLORIDE AND PHOSPHATE IN NORMAL SUBJECTS AND IN PATIENTS WITH ESSENTIAL ARTERIAL HYPERTENSION [1]

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The threshold theory of chloride excretion was formulated by Rehberg (1) in 1926. Chloride studies in recent years have included those of Dillon (2), Hare, Hare and Phillips (3), Shannon (4) and Wolf (5). Pitts in 1945 (6) considered chloride with reference to renal methods for the acidification of urine, and Wolf applied the law of exponential decay to his analysis of chloride disposal under varying infusion loads and at varying time intervals. He found certain "critical concentrations of infusion fluid and urine, at which neither solute nor water are retained from an infusion, relative to one another." This study is thus an extension of Rehberg's threshold studies, through the extremes of load, and confirms the impression of a "plasma concentration above which chloride is excreted relative to water, and below which chloride is retained relative to water." Wolf also finds a "distortion or departure from normal of the ratio of chloride to non-chloride space, induced by this load."

Our interest in chloride metabolism was stimulated by an observation made in the course of routine clearance studies done upon a group of patients suffering from essential arterial hypertension (7, 8). It was found that the clearance of chloride in such patients tended to parallel the urine output, whereas in normal subjects the chloride clearance remained within a certain range, regardless of the volume output. In a preliminary series, values of chloride clearance periods rose as high as 6.6 ml. per minute, with a urine output of 7.4 ml. per minute, while control subjects tended not to exceed values of 3.0 to 3.4 ml. per minute, even with a urinary flow of 11 to 12 ml. per minute. We then attempted to investigate the mechanism of this phenomenon and hence to arrive at an evaluation of its significance and implications.

The technique of multiple clearance was chosen because it supplied us with the filtration rate, the effective renal blood flow, and, by calculation of the U/P inulin, a helpful index to water reabsorption. It will be readily seen that the ratio of urine concentration to plasma concentration, or U/P inulin, is serviceable in 2 important respects, namely, that the complete filtration of inulin through the glomerular membrane is thoroughly established, and the concentration in the filtrate is known to equal that in the plasma; and secondly, that it is inert in the tubules. The U/P inulin can, therefore, be altered only by a change in the volume of the solvent. Such a change would, in the light of present concepts of renal physiology, be accomplished by reabsorption of water by the tubules. Thus, a U/P inulin of 100 indicates that the urine is 100 times as concentrated as the plasma, and that the corresponding volume of water has been reabsorbed from the glomerular filtrate. Likewise, if the U/P inulin is 1, then no water has been reabsorbed; the urine concentration equals the plasma concentration, and the urine flow is equal to the filtration rate.

Adopting, then, the inulin ratios as a measure of tubular reabsorption of water, and the range of those ratios as the pattern of renal behavior toward a substance which has no function in the body, the pattern for a threshold substance might now be predicted. Taking again, for example, a U/P inulin of 100, at which ratio 99 parts of water would have been reabsorbed, a threshold substance would show a smaller ratio, since the urine concentration, or the numerator, would have been modified not only by the 99 parts of water removed, but by the reabsorption to some degree of the solute. How much less the U/P ratio would be than that of inulin would depend upon the amount of the substance reabsorbed. Non-threshold substances might be expected to be less extensively reabsorbed than would threshold sub-

stances, but the strongest evidence of the character of the absorptive mechanism could be seen in the ability of the U/P ratios to fall below 1, since that situation could exist only if the tubules were able to reabsorb abundantly even at low U/P inulin ratios. Thus, if the U/P ratio of a given substance could sink below 1, it would be exceedingly difficult to wash that substance out of the body. If, in addition, such a substance could be shown to be reclaimed in defiance of decreasing tubular resorption of water, to the point where the U/P inulin is 1, and no water whatever were reabsorbed, then a U/P of 1 would complete the reabsorption gradient of a perfect threshold substance. Such a gradient would imply an exceedingly delicate and effective guardianship of an indispensable metabolite.

If, on the other hand, another substance were shown to have, at all U/P inulin ratios, a somewhat higher U/P ratio than did the threshold substance, but one still greatly lower than that of the totally inert polysaccharide, it would be deduced that a certain quantity had been reabsorbed from the glomerular filtrate, that quantity being less than that of the threshold substance. If this second substance were similarly calculated in terms of U/P and plotted against the U/P inulin, a figure of 1 at U/P inulin equals 1 would indicate that a urine unmodified toward inulin would also be unmodified toward such a non-threshold substance. The tubular cells would have reabsorbed neither water nor the metabolite in question which would, under these conditions, be as inert as inulin. If this were the case, it would be possible to wash such a substance out of the body, and the reabsorption gradient would be a graphic representation of a non-threshold substance.

In addition to clearance determinations of inulin and diodrast, chloride was chosen for analysis as a threshold substance, and phosphate as a probable non-threshold substance.

METHODS

Subjects were selected who, although complaining of "essential hypertension," gave no evidence of primary cardiac or renal disease. They varied in age from 19 to 60 years. The control group was drawn from other services in the hospital, and all subjects were found to be free from cardio-vascular or renal disease before they were included in the series.

The study was divided into 2 parts: the first being a

consideration of 2 random groups, having only the qualifications just described, the second being a protracted study of 2 individuals of the same sex and of comparable age, one of whom had essential hypertension of long duration, the other of whom had no demonstrable pathology of the heart, blood vessels or kidneys.

Concomitant clearance periods of inulin, diodrast, chloride, and phosphate were run on all subjects. All had the same type of diet, and were similarly hydrated prior to the tests which were performed in the fasting state. The clearance periods varied from 15 to 30 minutes in length, and the blood specimens were drawn in the first and third periods. The urine specimens were collected by catheter and the bladder was washed with sterile water, the washings being added to the collections. Blood samples for serum chlorides were drawn under oil, and analyzed by the method of Sendroy (9). Inulin determinations were performed by the method of Corcoran and Page (10).

Since the volume of infusion was small, and varied but slightly from case to case, the "load," as defined by Wolf, was negligible, and the subjects were regarded as in chloride balance. Since, too, the present method has had but little application, the behavior of the control group seemed to us worthy of particular analysis.

RESULTS

Repeated determinations performed on a normal subject indicated that U/P chloride is directly related to U/P inulin, and hence that the 2 values plotted against each other form a straight line. The same is true of a group of normal subjects, although the scatter was greater (Figure 1).

In the normal individual, as the U/P inulin decreased and the regression line approached a U/P inulin of 1, the U/P chloride fell below 1 and approached zero (Figure 2).

Phosphate values on the same individual, similarly plotted, also fell in a straight line, and the calculated regression line ran exactly through the point at which U/P inulin and U/P phosphate were 1 (Figure 3).

Comparing now the behavior of hypertensive subjects with the normal curves, Figure 1 shows that with rare overlapping determinations, the chloride ratio was higher at all inulin ratios in the experimental group than in the control group. Figure 2 demonstrates the same phenomenon in a single hypertensive subject when studied at varying rates of urine flow.

DISCUSSION

Returning to the threshold concept, let us examine the normal chloride curves in Figures 1
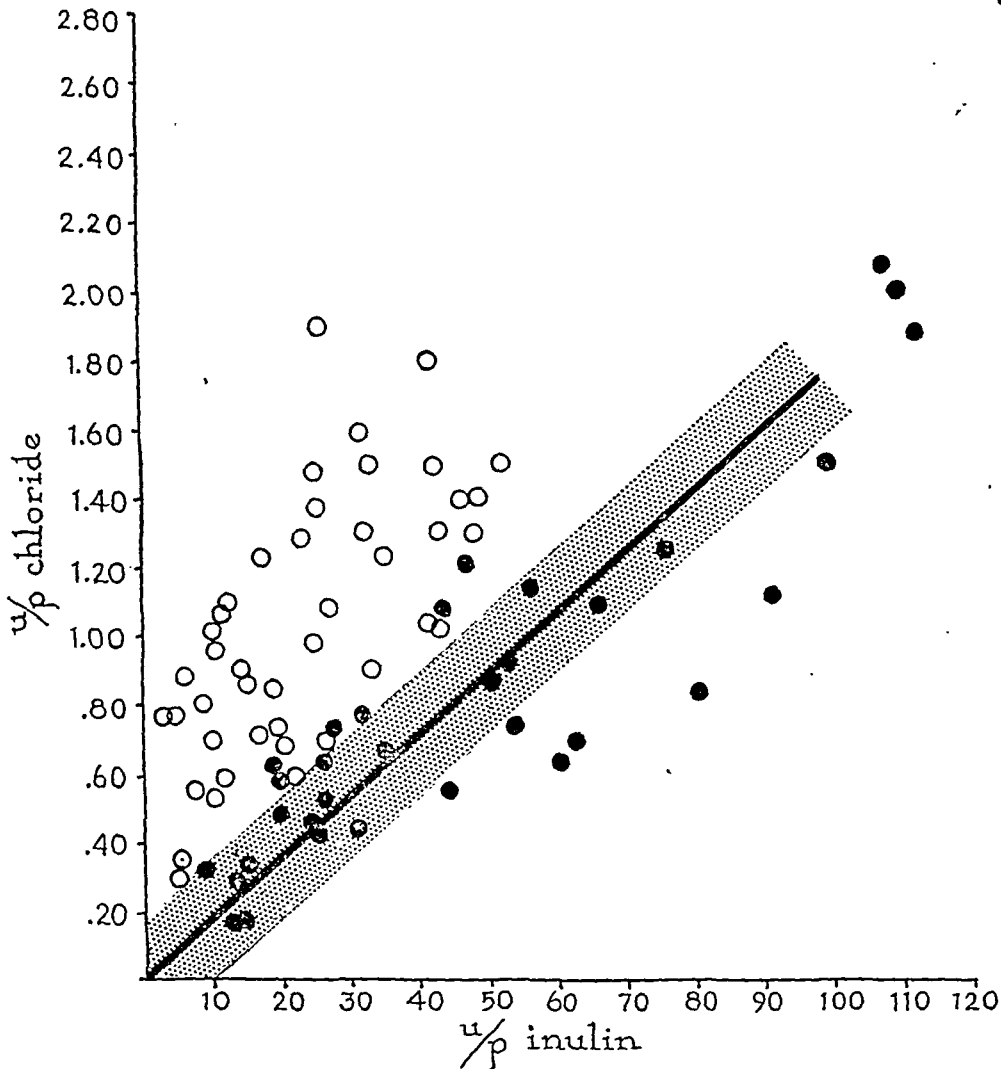
FIG. 1. U/P CHLORIDE IN TERMS OF U/P INULIN

Black symbols represent normal subjects; white symbols represent subjects with essential arterial hypertension. The coefficient of linear correlation for the normal group is: $r = 0.964$ with a probable error of ± 0.0082.

and 2 to see how these data may be brought into conformity with the threshold theory. In no instance was the plasma chloride elevated or depressed beyond normal limits; hence it may be argued that the theory may not be applied. In raising the urinary output by water diuresis, however, and lowering the U/P inulin, the hypochloremic state may be thought of as instantly recurring and being compensated, in the only fashion by which a decreased plasma chloride could occur without affecting other physiological factors to such an extent as to obscure the renal

dynamics toward chloride alone. At all outputs, then, the ratio of U/P chloride to U/P inulin was found to describe a constant proportion. Extrapolating for figures below those which are physiologically demonstrable, the regression line is seen to pass through U/P chloride ratios of decreasing fractions to a theoretical zero at U/P inulin unity. At this hypothetical end point the entire volume of glomerular filtrate would pass unmodified through the tubules, while all the chloride would be extracted and restored to the circulating plasma. Such a gradient appears to represent the pattern

of conservation practiced by the normal human subject in whom a water load is constantly pro- ducing a diminished plasma chloride concentration.

It is, perhaps, not out of order to add that one patient under treatment for Addison's disease was studied by the same method. The plasma chloride in this individual was 488 mgm. per cent. The ratio of U/P chloride to U/P inulin was found to be somewhat above the zone of estimate for our normal group. The chloride clearance was well within the normal range.

The finding of a constant proportion between reabsorption of chloride and that of water implies a very delicate balance between the inverse activi- ties of proximal and distal tubules, since chloride reabsorption is known to be carried on in the
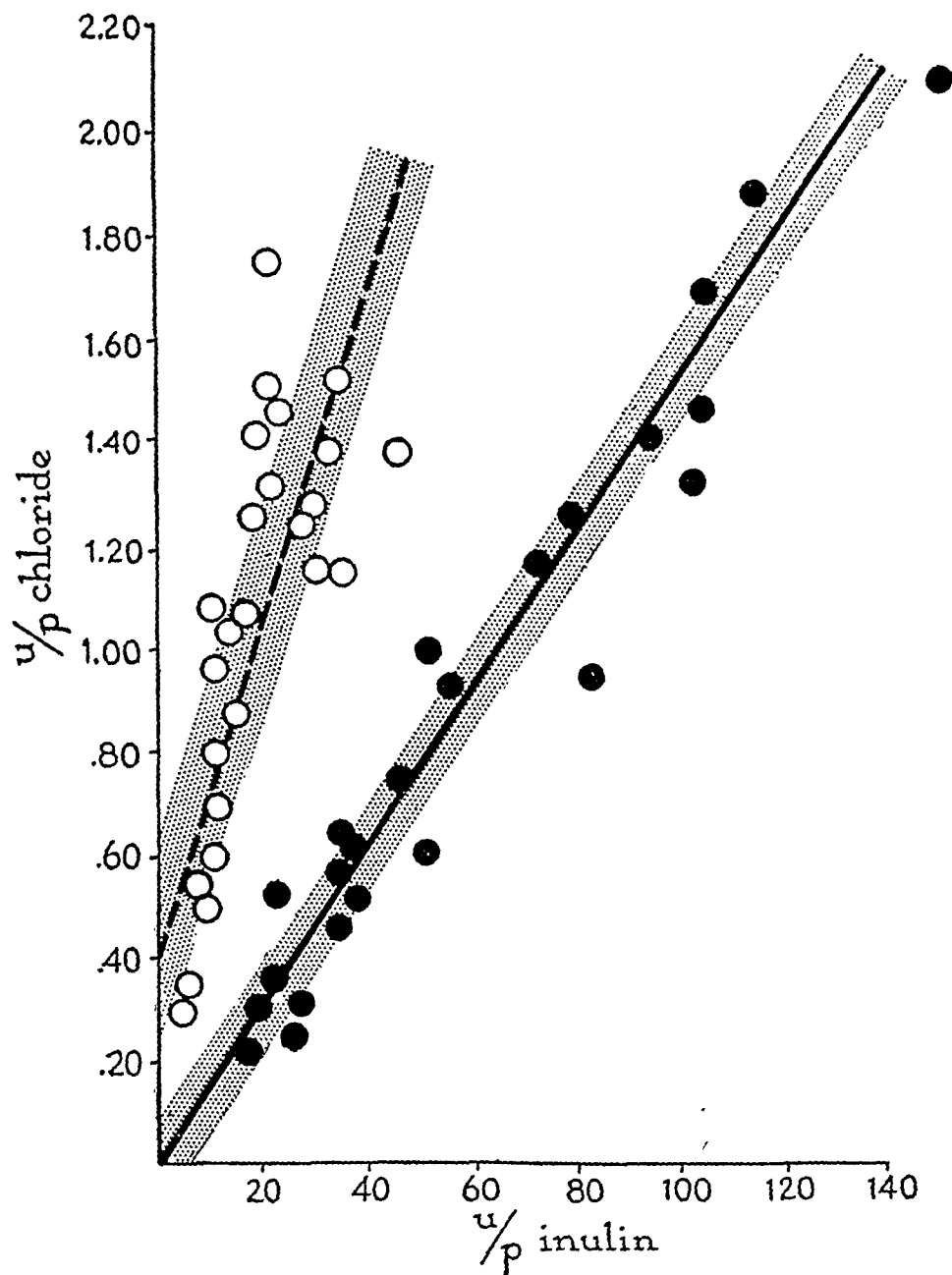
FIG. 2. U/P CHLORIDE IN TERMS OF U/P INULIN

Black symbols represent clearance periods obtained from a normal subject; white symbols represent clearance periods from a patient with essential arterial hypertension. The coefficient of linear correlation for the normal subject is .989 ± .0045 with a standard error of estimate of .09. The coefficient of linear correlation for the hypertensive patient is .894 ± .0261 with a standard error of estimate of .18.
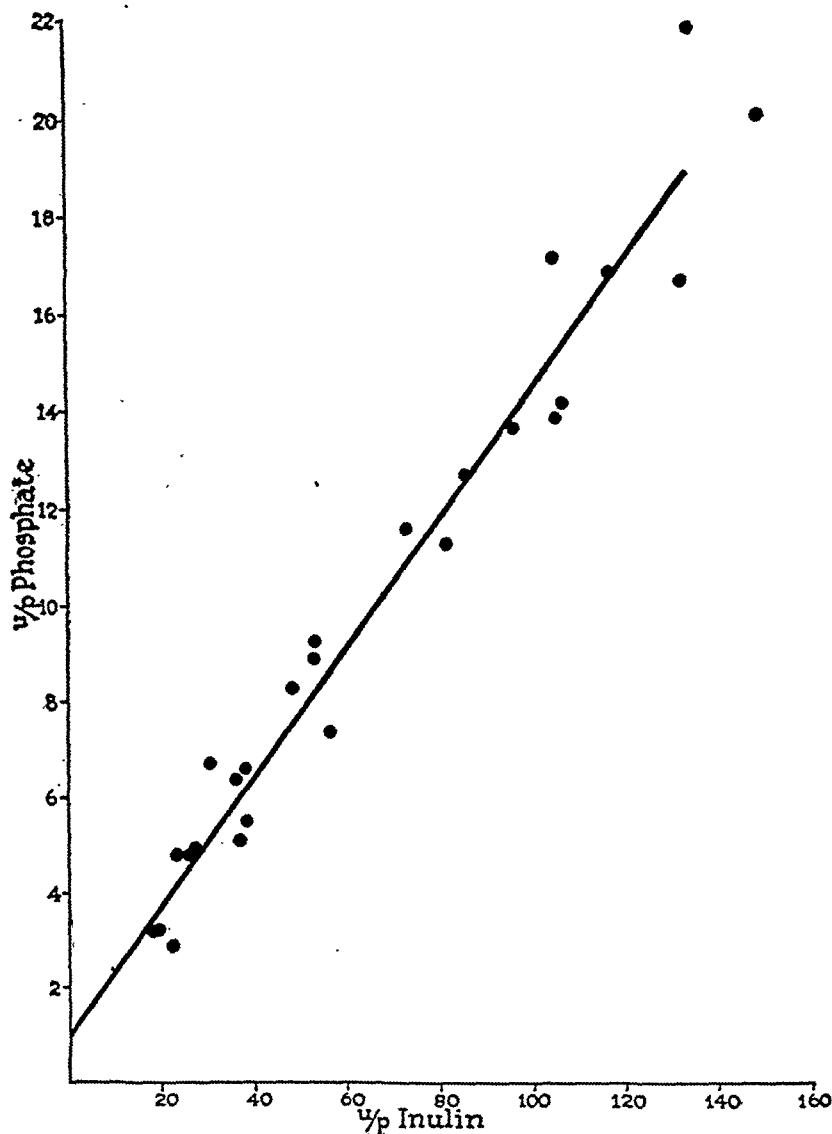
FIG. 3. U/P PHOSPHATE IN TERMS OF U/P INULIN

Symbols represent clearance periods obtained from the same normal subject.

proximal convolutions, while water is largely reabsorbed in the distal portions. Thus, while the proximal tubules are reabsorbing more chloride, the distal tubules are reabsorbing less water, the resultant of these operations remaining constant. The numerical ratio of this constant proportion evidently varies somewhat in different individuals, since the scatter is greater in the group study than in the individual; but even in the group the resultant regression line extrapolates to U/P inulin equals zero (or 1.0) and the ratio is hence always the same.

In contrast to the chloride situation, the U/P phosphate values (Figure 3) were found to run higher than the chloride ratios, although always lower than those of inulin itself. Here again, good correlation was found with U/P inulin, and a straight line curve resulted. Phosphate is, therefore, seen to be reabsorbed during its passage in solution through the tubules, but in diminishing proportion until, at the theoretical end point of U/P inulin unity, the U/P phosphate would also be unity, and the tubular cells would reabsorb neither water nor phosphate. Here again, is a

gradient, a non-threshold graph, similar to that of chloride, but resulting in opposite physiological accomplishment.

It is noteworthy that the U/P inulin values in the hypertensives are limited to a comparatively narrow range, extending from 4.8 to around 50, while normal subjects, under identical routine preparation, showed inulin ratios as high as 200. It is highly probable that this failure to reabsorb

water is simply an expression of hyposthenuria corresponding to degeneration of the distal tubules. If such be the case, this limitation of the U/P inulin range might well serve as a practicable clinical test, possibly employed with the single injection method.

Turning now to the data as they pertain to the hypertensive patients. Tables I and II show the clearance of chloride running materially higher in

TABLE I

Data obtained from a group of patients with essential arterial hypertension

	B.P.	ml. per min.	$C_{In.}$	$C_{Diod.}$	$C_{Chlor.}$	FF	U/P Chlor.	U/P In.
C. V.	210/140	2.5	57.2	295.3	3.4	.220	1.47	24.9
		5.7	67.2	305.5	5.0		1.07	11.7
		6.0	65.0	256.9	5.8		.97	10.8
	190/120	11.6	66.3	176.0	4.0	.389	.34	5.7
		5.8	65.0	165.9	3.3		.58	11.3
		4.2	64.1	160.2	3.6		.87	15.4
	228/120	9.7	67.4	345.4			.31	5.0
		12.7	60.6	271.8	4.5	.204	.54	7.7
		6.7	58.0	298.5	3.6			
	210/126	6.8	61.9	291.7	3.4	.213	.52	9.7
		6.1	61.3	266.6	4.1		.69	10.1
W. G.	220/134	9.4	92.5	337.1	6.5	.284	.69	9.8
		6.7	94.5	316.1	6.0		.90	14.3
		5.4	92.8	335.6	7.4		1.23	17.1
	180/110	4.6	98.0	328.0	2.8	.299	.59	21.1
		3.3	85.0	384.0	2.3		.70	27.0
C. S.	184/132	4.6	77.0	514.4	3.0	.187	.69	16.9
		6.0	107.2	603.9	3.9		.71	18.1
	158/112	4.7	63.7	420.0	3.9	.149	1.10	12.1
		2.9	73.8	422.2	5.7		2.10	26.7
	184/120	3.4	66.2	300.0	3.0	.249	.86	18.8
		2.9	76.6	328.0	3.1		1.09	26.8
		4.0	103.0	357.0	4.0		.99	25.6
M. S.	144/ 86	6.7	135.6	795.6	4.5	.183	.68	20.2
		7.1	133.2	729.9	5.2		.73	18.7
		7.0	134.0	679.6	4.9		.69	19.2
L. McG.	240/140	7.1	72.4	328.0	6.6	.255	.93	10.2
		8.0	66.2	246.6	6.4		.80	8.3
E. L.	200/138	4.9	75.1		4.1		.88	15.2
		1.8	76.8		3.7		2.00	42.7
E. A.	175/105	1.4	49.9	306.0	1.8	.169	1.24	35.1
		2.6	67.4	405.0	2.6		1.00	26.4
		2.8	94.7	548.0	3.5		1.22	33.6
S. D.	202/112	5.1	31.0	125.0	6.0	.282	.90	6.0
		9.1	43.3	151.0	6.9		.77	4.8
		8.5	46.5	152.0	6.5		.77	5.5
V. D.	160/110	3.5	88.7	459.0	4.9	.208	1.37	25.4
		3.3	101.0	460.0	5.1		1.59	31.8
		4.2	96.5	458.0	5.2		1.29	23.0

TABLE II

Data obtained from a single patient with essential arterial hypertension

	B.P.	ml. per min.	$C_{In.}$	$C_{Diod.}$	$C_{Chlor.}$	FF	U/P Chlor·	U/P In.
C. V.	200/105	2.0	68.9	253.0	3.0	.301	1.52	35.0
		2.0	61.2	205.0	2.3		1.16	31.4
		2.1	74.3	233.0	2.4		1.16	35.5
		1.0	45.3	139.0	1.4		1.39	47.0
	210/110	2.0	60.4	261.0	2.5	.202	1.25	29.2
		1.7	52.0	242.5	2.2		1.27	30.4
		1.6	55.6	246.5	2.2		1.42	35.5
		1.4	46.7	234.0	1.9		1.38	34.1
	208/108	3.8	58.0	220.0	3.9	.226	1.05	15.3
		2.5	53.0	218.5	3.5		1.43	21.4
		1.9	41.6	225.2	3.3		1.75	22.1
		2.2	49.0	228.0	2.9		1.32	22.3
	228/125	4.3	52.0	256.0	3.5	.231	.80	12.0
		2.8	57.2	247.5	3.5		1.27	20.5
		4.1	64.8	271.7	4.2		1.06	16.3
		2.7	62.6	247.8	4.1		1.50	23.1
	228/120	9.7	67.4	345.4		.204		
		12.9	60.6	271.8	4.5		.31	5.0
		9.8	58.0	298.5	3.6		.54	7.7
	210/140	2.3	57.2	295.3	3.4	.220	1.47	24.9
		5.7	67.2	305.5	5.0		1.07	11.7
		6.0	65.0	256.9	5.8		0.97	10.8
	190/120	11.6	66.3	176.0	4.0	.389	.34	5.7
		5.8	65.0	165.9	3.3		.58	11.3
		4.2	64.1	160.2	3.6		.87	15.4

that group than in the control (Tables III and IV). Although a strict correlation is not found between the chloride clearance and the urine output, the higher outputs are more apt to show high clearances. In the normals, however, the output volume has clearly no relation to the clearance of chloride. Converting to ratios, Figure 2 shows a different regression line with no overlapping, in the case of the hypertensive patient. Calculation of the regression line of this curve shows a positive intercept on the chloride scale, indicating that in this situation a constant proportion no longer exists, and that at U/P inulin equals 1, chloride is not being completely reabsorbed, but is passing out in the urine in concentration roughly equivalent to ½ of plasma concentration. In view of the fact that the hypertensive data constitute not so much an absolute pattern as a derangement in varying degrees of a pattern, the definition and extrapolation of a regression line is of questionable value. For this reason, the statistical treatment of the hypertensive data in Figure 1 has been omitted, and the discussion is based rather on the observation that at all U/P inulin ratios, the hypertensive patients reabsorb less chloride than do the normal subjects. Thus, not only do the tubules fail to reabsorb water, but, per unit of water reabsorbed, they demonstrate a specific failure to reabsorb chloride, with the result that at all dilutions the hyposthenuric output contains several times more chloride than normal.

The interpretation of these findings with respect to disposal of chloride by the kidney in hypertension is highly problematical. If the chloride clearance (Tables I and II) tends to vary with the urine volume, and the normal chloride economy gives evidence of impairment with resulting seepage of chloride in the urine, a chronic condition of relative chloride want might well be suspected. The scope of the present study does not permit more than conjecture as to the reasons for the reduced reabsorption which might be on a basis of adrenal cortical insufficiency, excess pitocin, or functional impairment of the proximal tubules. Whatever be the true explanation, such a modification of behavior toward chloride appears to be a specific

TABLE III

Data obtained from a group of normal subjects

	B.P.	ml. per min.	$c_{In.}$	$c_{Diod.}$	$c_{Chlor.}$	FF	U/P Chlor.	U/P In.
L. H.		4.4	109.0	608	2.0	.185	.45	24.9
		4.3	109.0	573	2.3		.52	25.6
		3.6	94.7	501	2.3		.63	26.3
J. S.		1.7	104.0	548	1.1	.189	.69	62.8
		1.2	95.8	510	1.0		.83	80.5
		1.7	105.0	543	1.1		.64	60.0
M. S.		5.7	72.2	357	1.6	.201	.28	12.8
		2.5	79.0	398	1.9		.77	31.6
		2.7	72.2	346	1.9		.72	27.2
E. H.		1.2	85.0	605	1.3	.149	1.14	56.0
		1.1	84.2	549	1.3		1.22	76.5
		2.6	78.6	506	1.1		.44	30.7
M. E.		8.1	121.0	658	1.7	.221	.17	14.8
		3.3	145.0	638	1.9		.56	43.6
		2.6	124.0	497	1.7		.74	52.3
I. F.		3.9	76.0	446	2.3	.180	.59	19.6
		3.8	74.3	361	1.8		.47	19.6
		.5	76.3	447	1.4		2.84	124.0
M. M.		6.9	93.6	463	1.1	.187	.16	13.6
		2.8	68.9	338	1.3		.42	24.4
		.8	80.8	504	1.6		2.06	106.0
C. H.		1.6	118.0	508	1.8	.211	.91	52.7
		2.7	136.0	690	2.3		.87	50.4
V. M.		2.3	82.7	374	1.5	.238	.65	35.0
		1.5	96.4	421	1.6		1.08	66.0
		1.2	112.0	427	1.7		1.51	98.2
M. D.		1.2	134.6	715	2.2	.181	1.87	111.2
		1.8	194.2	1104	3.6		2.00	108.5
T. B.		1.9	83.0	452	2.1	.183	1.07	43.3
		1.8	85.2	483	2.2		1.21	47.5
		2.1	92.7	492	2.3		1.07	43.7
D. R.		8.6	76.7	430	2.7	.186	.31	8.9
		7.7	83.8	460	2.5		.33	14.7
		3.9	69.0	347	2.3		.63	18.6

characteristic inherent in the tubules, or in which the tubular epithelium acts as the agent.

SUMMARY AND CONCLUSIONS

Studies of the filtration rate, renal blood flow and chloride excretion were performed by means of concomitant clearance tests of inulin, diodrast and chloride upon: (a) a group of normal individuals and (b) a group of patients with "essential hypertension." By the same method protracted studies were done of: (a) a single individual without vascular or renal disease and (b) a patient of corresponding age and sex with well established hypertension.

Tabulation of chloride clearance and urine flow in these groups showed a fixed range of chloride clearance independent of the urine flow in the normal individuals. In the hypertensive patients the chloride clearance tended to vary directly with the urine flow.

Plotting of U/P chloride against U/P inulin in the normal cases resulted in a straight line relationship of constant proportion, which in the group study, as in the individual, dropped below U/P chloride values of 1 and approached zero. It was, therefore, demonstrated that under conditions of extreme diuresis, the tubular epithelium actively retracted the chloride from the filtrate, with the

TABLE IV
Data obtained from a single normal subject

	B.P.	ml. per min.	$c_{In.}$	$c_{Diod.}$	$c_{Chlor.}$	FF	U/P Chlor.	U/P In.
J. P.		2.9	106.0	596	1.9	.199	.7	36.4
		1.7	85.0	400	1.3		.8	48.4
		2.3	85.5	418	1.2		.5	37.8
		0.8	85.2	400	1.1		1.3	103.0
		3.3	74.5	412	1.2	.190	.4	22.3
		2.2	81.0	420	1.3		.6	36.9
		4.1	78.0	403	1.3		.3	18.9
		3.0	78.9	407	1.3		.3	26.1
		0.7	86.5		1.4		2.1	130.0
		0.7	77.9		1.3		1.7	105.0
		0.3	68.2		1.1		3.9	253.0
		1.4	.75.5		1.4		1.0	53.0
		1.2	68.2	366	1.2	.180	.9	56.1
		1.0	78.4	425	1.2		1.3	80.5
		0.7	76.6	423	1.3		1.9	115.0
		2.4	91.5	534	1.5		.6	38.3
		3.6	82.1	464	1.9	.187	.5	22.8
		0.8	78.6	418	1.1		1.5	104.0
		0.9	82.5	430	1.2		1.4	95.5
		0.9	69.5	360	1.1		1.2	72.5
		1.4	51.6	276	0.7	.173	.5	35.5
		4.1	75.4	440	0.9		.2	18.6
		3.1	86.6	516	1.0		.3	27.6
		1.5	79.4	395	0.5	.218	.3	52.5
		1.0	87.6	378	0.5		.5	84.7
		0.6	91.5	386	0.5		.9	146.0
		0.7	92.4	445	0.6		.9	131.0

theoretical end-result that if no water whatever were reabsorbed in the tubules, all chloride would be reabsorbed, and the urine would be a chloride-free filtrate unmodified with respect to inulin. In order to explore the application of this method of analysis, phosphate values on the same case were similarly calculated and graphed. Phosphate was found to behave like a non-threshold substance, the U/P ratio of which declined with the U/P inulin to a theoretical value of 1.

The U/P chloride in both group and individual hypertension studies was higher at all U/P inulin ratios than the normal curve. Such an alteration in behavior toward chloride represents an impairment of the normal reabsorption mechanism.

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PROCEEDINGS OF THE THIRTY-EIGHTH ANNUAL MEETING OF
THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION
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READ BEFORE THE SCIENTIFIC SESSION

PRESIDENTIAL ADDRESS

BIOLOGICAL BEACHHEADS

By THOMAS FRANCIS, JR.

In the past year a group of nations of divergent ideologies, through coordinated and exhausting effort, has succeeded in applying control measures to a disease which had been increasing in scope and virulence so as to reach epidemic proportions in the world politic. A few experienced political epidemiologists had recognized the danger early and pointed out the threat, but the majority relied upon our isolation and quarantine regulations to meet it. Various placebos were tried. It was not, however, until an open focus appeared on our own flank that we really rose to action. The emergency demanded highly concentrated authority, enormous expenditures and the drafting of men from all activities into one great effort. By amazing combinations of land, sea and air power, beachheads were established and a *cordon sanitaire* formed. From these points the further advances were made which finally disrupted the essential reservoirs to a point of ineffectiveness, and the epidemic subsided.

Like all other epidemic diseases, however, it survives in foci to which must be applied all conceivable specific and environmental control measures in order to prevent a resurgence. This is the foremost problem of the day and, unless effectively approached, reduces our other hopes for advancement to sheer vapor.

No people can pass through such experiences without receiving a deep imprint upon its philosophy. The influence extends beyond the social and political fields, hence, it is not surprising that the patterns which were developed under military exigencies should be proposed as those which would function effectively in the establishment of biological beachheads.

The trends are seen in the current discussions of national legislation in support of research. One group has urged action similar to the methods used in the atomic bomb research project. It apparently visualizes a higher echelon of master minds which plans, organizes and directs—busily splitting its mental 235 in an effort to set up a chain reaction in the low grade cerebral pitchblende of a large body of workers. Research workers are human tools—the important thing is to know what job they are good for and how to use them. The impression is promoted that the mass attack will settle problems of disease quickly and finally. Another proposal accepts the desirability of increased funds for scientific endeavor from federal appropriations to be distributed by a body of scientists with a minimum of governmental direction—but nonetheless an organization administered through the federal government.

There is general agreement that it is highly desirable

to encourage more men of ability to enter the different fields of scientific investigation. Moreover, if these men can develop sufficient common interest to study certain problems with different disciplines and different points of view, to share experiences and trade ideas, it is clearly advantageous. The past five years have furnished numerous examples of men giving up their individual preferences to join in a coordinated effort designed to test a lead or to give their abilities to continued, tedious studies, even though disagreeing with the major plan. Furthermore, in most problems a stage is reached where it is profitable and strategic to concentrate upon an organized study which can test under controlled conditions the validity and applicability of experimental observations. This quantitative approach would seem to be most useful when the stage of application is reached. But whether the concept of coordinated study can be expanded indefinitely is open to considerable doubt.

Disease is a complex biological problem and while much has been learned concerning certain mechanisms at play and the reagents involved, I believe it is fair to say that the intimate details of but few of them are really known. Nor, with possibly a few exceptions, is it clear how a clinical case of disease actually begins. While we have reached the atomic age in the physical sciences, biologically we are still in the molecular stage. Experimental work is primarily qualitative in its approach. It constitutes the advance intelligence, the scientific G-2, seeking to gather data on which biological beachheads can be established. It requires judgment in evaluating and correlating small bits of information and in deciding which areas are most vulnerable to attack. It must not allow rumor in the form of uncontrolled observations, or premature interpretation to be mistaken for fact. "The experimental method," said Claude Bernard, "is the scientific method which proclaims freedom of mind and of thought." It does not submit to authority, but draws from within itself an impersonal authority which dominates science. Original thinking and observation do not derive from conformity with accepted opinion.

How best can a national research foundation serve to promote these ends and at the same time preserve spontaneity and freedom of action? Certainly, not through another atomic bomb project. Perhaps the greatest reservations concerning the desirability of a national agency lie in skepticism as to the spirit in which it will function. Administrative principles of a large organization tend to be founded on orthodoxy. There should be as little display of central authority and direction as

can possibly be got along with. Care should be taken to avoid the setting up of favored programs of research to the exclusion or disadvantage of other less prominent fields of activity; this procedure could lead to lobbying and pressure groups who would work to promote special interests. Moreover, programs incline inevitably to seek the application. One might, with a certain sincerity, suggest the motto "A pogrom on programs." The current tendency to give support only to well defined projects should be minimized, and encouragement given both to extended work in a broad field which offers many opportunities, and to dabbling in a narrow field where the next move is obscure. Gilding of the lily should not become the major scientific objective; the simplicity of observation should not give way to the glamorous phrase; the seat of influence must not replace the work bench in determining the direction of investigation. A national foundation must be free of political domination by either the professional or the scientific politician. If undertaken it must be inaugurated on a sufficiently sympathetic basis that it shall not have to justify itself in terms of the first few years' results, nor concern itself too greatly with detailed evaluations of the progress which is being made. The concept which flows through the Bush report of general research funds largely administered through university channels to meet their own needs, and to take advantage of their own resources, seems of all the most desirable, because it returns the opportunity and the responsibility for initiation and support of research to its proper place. Moreover, it would probably result in a great reduction in incidence of that occupational disease of the experimentalist, a divergent strabismus developed from trying to keep one eye on his work and the other on the source of funds.

There is a need to increase the positions and the environments in which men can work continuously upon problems in which they themselves are interested. The current system contrives to drive men from research since the positions to which they can advance furnish frustration rather than fruition of their research interests. The generation of ideas and new information should constitute sufficient warrant for their support without detailed pleading before a dispensing board.

There is a need to promote the investigation of disease as a broad problem in biology. Too often the preclinical years of the medical curriculum are urged to emphasize clinical application rather than biological implications. Greater latitude for thought and work in these latter channels will undoubtedly extend the boundaries of clinical investigation.

There is little doubt that if more men who could be interested in the investigation of disease problems were found, given the opportunity to develop, provided facilities and *time*—for thought and work, afforded reasonable incomes and opportunity to advance, all in an atmosphere of scientific freedom, progress in medicine might well be more rapid. This is the manner in which increased funds can be most effectively employed.

In the original formation of a federal organization for the support of scientific research, which now seems imminent, it will be the responsibility of members of this and other research societies to see that the personnel and the philosophy upon which they act are truly representative of the best scientific thought. Otherwise it is we, not they, who will have lost the beachhead. Failure will not only be harmful to *research,* but will destroy a great opportunity science has had to project the honesty and objectivity of the scientific outlook into a world of social confusions and warped emotions.

A Comparison Between Infectious Hepatitis and Serum Jaundice in Experimentally Infected Human Volunteers. By W. PAUL HAVENS, JR., New Haven, Conn.

Although the exact relationship between *infectious hepatitis* and *homologous serum jaundice* is not understood, experiments in the transmission of these two conditions to human volunteers have revealed certain information which assists in making a distinction between them.

The causative viruses of both diseases are filtrable, resistant to a temperature of 56° C. for 30 minutes, and may be transmitted to human volunteers in serial passage, producing a clinical disease in man which resembles catarrhal jaundice.

In contrast to these similarities are certain differences which include route of infection, length of incubation period, and period of infectivity.

Our strain of *infectious hepatitis* virus produces disease in human volunteers following parenteral inoculation or ingestion, with incubation periods ranging from 15 to 34 days. Virus is demonstrable in both blood and stool during the acute phase but not in the incubation or convalescent periods. Homologous immunity is present in these patients.

Our strain of *homologous serum jaundice* is infectious when inoculated parenterally with incubation periods ranging from 56 to 134 days. It is demonstrable in the blood of inoculated subjects one-third and three-fourths way through the long incubation period and during the acute phase of disease, but not 1 month after onset. Apparently it is not present in the stool of patients, nor is it infectious when ingested. Patients convalescent 6 months from infection with this strain are not immune to experimental infection with our strain of *infectious hepatitis* virus.

A Study of the Urinary Coproporphyrin in Hepatitis and Cirrhosis. By C. J. WATSON, Minneapolis, Minn.

In this study the total coproporphyrin in the 24-hour urine was determined in 154 cases. This series included 60 cases of infectious hepatitis; 45 cases of cirrhosis of the liver of various types; 17 cases of jaundice due to extrahepatic biliary obstruction; 1 case of carbon tetrachloride poisoning with jaundice; 1 case of jaundice due to sulfanilamide. The relative percentage of the type 1 and 3 coproporphyrin isomers was determined by means of differential precipitation of the esters in 30 per cent acetone, as described by Schwartz, Hawkinson, and Watson. Normal human urine has been found to contain from 65 to 90 per cent of the type 1 isomer. The normal range for the total coproporphyrin in 24 hours

is from 30 to 100 gamma, most often from 40 to 80 gamma.

The present study permits the following conclusions:

1. The urinary coproporphyrin is commonly elevated in acute hepatitis. The elevated values persist well into the defervescent period and in many instances, for some time after other liver function tests have returned to normal or near normal range. The increase in all instances thus far studied has been due to an increase in the type 1 isomer. One possible exception has been encountered in a case of sporadic jaundice in an individual who was a moderate alcoholic and who had been exposed to other chemicals. In this case, 90 per cent of the coproporphyrin in the urine was the type 3 isomer. In the case of jaundice due to carbon tetrachloride poisoning and to sulfanilamide, the increase of coproporphyrin was the type 3 isomer.

2. The total coproporphyrin of the urine is often increased in cases of cirrhosis of the liver, markedly elevated values being noted in some cases. Elevations of the same magnitude are commonly encountered in jaundice due to extrahepatic biliary obstruction. Thus, the determination of the total coproporphyrin has but slight value in the differential diagnosis of jaundice. Of chief interest, however, is the finding that in the majority of cases of cirrhosis in alcoholics, the elevated coproporphyrin value is due to increase of the type 3 isomer while in the remainder, including the cases following infectious hepatitis, the increase has always been of the type 1 isomer.

The Mechanisms of the Liver and Kidney Injury Produced by Toxic Substances. I. Some Effects of Pyridine and Their Prevention by Methionine. By JAMES H. BAXTER (introduced by Tinsley R. Harrison), Dallas, Texas.

Experimental liver and kidney injury including cirrhosis have been produced in two general ways: by administration of toxic substances and by feeding diets deficient in lipotropic substances. This investigation deals with the relationship between the processes which follow.

When pyridine was added to an otherwise adequate diet and fed to young rats, weight gain ceased and death occurred in about a week. Livers and kidneys showed extensive degenerative changes. Animals surviving for longer periods developed cirrhosis.

The addition of methionine to the diet prevented these effects to a considerable extent.

Pyridine was used in these experiments because it is methylated in the animal body (at least in dog and man), probably at the expense of lipotropic substances which act as methyl donors.

There is also evidence that a number of other hepatotoxic and nephrotoxic substances may be methylated in the body, and still others may act by interfering with the action of lipotropic substances, perhaps by inhibition of enzyme systems.

Action of pyridine through other mechanisms which are influenced by certain substances containing sulfhydryl groups may be at least partially responsible for the effects observed.

If the liver and kidney damage produced by pyridine (and perhaps these other substances) prove to be due to this exhaustion of lipotropic factors, thus producing the same effect as a deficient intake of lipotropic substances, then, at least in some cases, "toxic" cirrhosis and "deficiency" cirrhosis may be fundamentally related.*

The effects of feeding methyl pyridinium hydroxide have been compared with the effects of feeding pyridine in stoichiometric amounts. If the toxic effects of pyridine are due to its methylation with exhaustion of lipotropic substances, then methyl pyridinium hydroxide which is formed by the methylation of pyridine would not be expected to produce these effects. Young rats continued to grow (though at a somewhat slower rate than normal) without apparent illness and without demonstrable lesions at autopsy, when given diets containing methyl pyridinium chloride equivalent on a stoichiometric basis to twice the concentration of pyridine necessary to produce death with extensive degenerative changes in the livers and kidneys.

Attempts to isolate methyl pyridinium hydroxide from the urine of rats fed pyridine are in progress, but so far have not been accomplished with certainty.

It should be emphasized that it is not intended to suggest that all the toxic effects of pyridine are due to the mechanism described, but only the liver and kidney injury and possible injury to certain other organs that have not been extensively studied. It is interesting that while pyridine produces depression of the nervous system and anesthesia, methyl pyridinium hydroxide in large doses is a convulsant. It is also interesting that when given intraperitoneally in mice, the M. L. D. of methyl pyridinium chloride is about 0.22 mgm. per gram, while the M. L. D. of pyridine is about 1.2 mgm. per gram or about eight times as much on a stoichiometric basis, despite the fact that the chronic toxicity of pyridine on oral administration is much greater than that of the methyl derivative.

Other toxic substances that might act through a similar mechanism will be investigated. Preliminary experiments indicate that cystine affords considerable protection against pyridine, but less than methionine. Choline alone has little or no protective effect, but choline plus cystine is more effective than cystine alone, and even more effective than methionine. Thioglycollate is ineffective while thiouracil gives marked protection.

The Coagulation Properties of Normal and Hemophilic Whole Blood, Plasma, and Plasma Protein Fractions. By JESSICA H. LEWIS (by invitation), GEORGE R. MINOT, J. P. SOULIER (by invitation), H. J. TAGNON, C. S. DAVIDSON, and F. H. L. TAYLOR (by invitation), Boston, Mass.

Normal and hemophilic whole blood, plasma and plasma protein fractions were studied *in vitro* and *in vivo*. The

* Explanatory Note: These studies are being extended in collaboration with Dr. Morton F. Mason.

prothrombin and fibrinogen content were found to be the same. The protease activity after treatment with chloroform, as measured by the nonprotein nitrogen production from a casein substrate and by lysis of a fibrin clot, was found to be similar in both hemophilic and normal plasma and globulin fractions. The electrophoretic pattern of hemophilic plasma did not differ measurably from normal plasma.

Shortening of the coagulation time of blood from patients with hemophilia was produced *in vitro* by the addition of small amounts of normal blood, plasma and certain globulin fractions; notably Fractions I, II + III, and IV_1 of Cohn. Fractions IV, 3 + 4 and V (albumin) had no effect. Globulin fractions prepared in a similar fashion from pooled hemophilic plasma had no *in vitro* shortening effect upon the coagulation time of hemophilic blood.

Transfusions of normal blood, plasma or Fraction I markedly shortened the coagulation time of hemophilic patients. Transfusions of similar amounts of hemophilic whole blood, plasma or Fraction I, derived from pooled hemophilic plasma, did not shorten the clotting time in hemophilia.

Thus, the difference in coagulation properties between hemophilic and normal blood was found not in the prothrombin, fibrinogen or protease activity, but in the fact that globulin derivatives of normal plasma have antihemophilic activity, while those derivatives from hemophilic plasma have no such activity.

Role of Synthetic Folic Acid in Blood Maturation. By TOM D. SPIES, Birmingham, Ala.

Our studies have shown that synthetic folic acid has a profound effect on the bone marrow of persons with Addisonian pernicious anemia, sprue, and related anemias. These investigations have been conducted in selected persons after complete history, physical examination, peripheral blood and bone marrow studies.

In selecting the cases, the following criteria were used: (1) The patient must have a macrocytic anemia with red blood cell counts of 2.5 million or less and a color index of over 1. (2) He must be untreated, or he must not have been treated recently enough to interfere in any way with our evaluation of the effect of folic acid. (3) He must not have any complicating disease which might be lethal during the course of the study. (4) The bone marrow must contain megaloblasts and have the typical erythroblastic arrest seen in macrocytic anemia. A fifth criterion used for the selection of any patient in Cuba, where we were studying sprue, was that he must have "fatty stools" and weight loss.

After the subjects were selected, they were placed on a control diet free of meat, meat products, fish, and poultry, and baseline determinations of blood values were made. Without any other alteration of the baseline conditions, synthetic folic acid was given either parenterally or orally in amounts ranging from 1 mgm. per day to 500 mgm. per day. Great clinical improvement usually begins around the third or fourth day, and this is corroborated by laboratory studies. The person feels stronger and develops an appetite, and the reticulocytes increase in the peripheral blood and in the bone marrow. Within two weeks the white blood cells, the red blood cells, and the platelets all increase in the peripheral blood, and the bone marrow tends to revert to normal. These findings, the dosages, the best methods of administration, and the mechanisms concerned will be discussed in detail.

Further Observations on the Anti-Pernicious Anemia Effect of Synthetic L. Casei Factor. By CARL V. MOORE and (by invitation) OLGA S. BIERBAUM, St. Louis, Mo.

The following observations have been made on the therapeutic effectiveness and mode of action of synthetic *L. casei* factor in Addisonian pernicious anemia.

1. Maximal or near-maximal reticulocyte responses have been obtained with daily doses as small as 1 mgm. given parenterally, and 3 mgms. given orally. These quantities do not regularly produce comparable responses, however, and there apparently is significant variation in minimal effective dose to which different patients will react.

2. *L. casei* factor can be absorbed from the rectum. When 100 mgm. were given daily as a retention enema to one patient, a nearly maximal reticulocytosis resulted.

3. Simultaneous administration of normal human gastric juice did not enhance the anti-anemic effectiveness of *L. casei* factor in one subject. This supports published evidence that the vitamin does not possess extrinsic factor activity.

4. Hematologic and clinical remissions, initially induced by *L. casei* factor, have been maintained for 6 months in 3 patients by the weekly injection of 140 mgm. of the synthetic material.

5. Hematologic and clinical remissions have been maintained for 6 months in 25 subjects, who formerly had been treated with liver extracts, by parenteral administration of 25 to 75 mgm., *L. casei* factor.

6. Manifestations of combined system disease have become worse in only one patient, and in this instance the change followed the development of cellulitis.

Studies on the Effect of Methyl Bis (β chloroethyl) amine hydrochloride * on Diseases of the Hemopoietic System. By LEON O. JACOBSON and (by invitation) CHARLES L. SPURR, E. S. GUZMAN BARRON, TAYLOR R. SMITH, CLARENCE LUSHBAUGH and GEORGE F. DICK, Chicago, Ill.

The therapeutic efficacy of intravenous injections of methyl bis (β chloroethyl) amine hydrochloride (Dema) in the treatment of certain diseases of the hemopoietic system has been studied. The diseases each of which

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* Abbreviated to "Dema" for purposes of brevity.

were confirmed by biopsy or sternal aspiration include:

Diagnosis	Number of patients
Hodgkin's Disease	25
Lymphosarcoma	6
Sympathicoblastoma	2
Multiple myeloma	2
Myelogenous leukemia	
Acute	1
Chronic	6
Polycythemia rubra vera	3
Lymphatic leukemia	
Acute	1
Chronic	7.

The dose of Dema used in this series of cases was 0.1 mgm. per kilogram of body weight and was given in courses of 1 to 7 daily injections. Ten mgm. of Dema were dissolved in 10 ml. of 0.9 per cent NaCl immediately before use and the calculated amount injected directly through the rubber tubing of a Fenwal intravenous medication set which was already delivering normal saline to the subject at a relatively rapid rate.

A delayed but extremely serious toxic manifestation is the lymphopenia, neutropenia and thrombocytopenia which develops and reaches a maximum within the first three weeks. The changes occurring in the peripheral blood are paralleled in the bone marrow. Sternal punctures indicate that an extreme degree of destruction may occur. Recovery, however, is complete after varying intervals.

The period of observation of the patients after single or repeated courses of Dema varies from 1 to 28 months. In all cases so treated except for the cases of acute leukemia, multiple myeloma and one case of lymphosarcoma, definite clinical remissions of the disease process involved were produced varying in duration from 1 to 8 months. The most encouraging results have been seen in Hodgkin's disease; symptoms were quickly alleviated and evidence of lymphadenopathy, splenomegaly and hepatomegaly regresses remarkably. Roentgenographic evidence of mediastinal enlargement and involvement of the parenchyma and hilum of the lung showed similar regression. Repeated courses of treatment with Dema have produced further remissions.

Superiority of this type of treatment over that of X-ray therapy is *not claimed* but cases with apparent X-ray resistance have responded favorably. Dema or a related compound may prove to be a useful adjunct to X-ray therapy.

The Treatment of Arsenic and Mercury Poisoning with BAL (2,3-dimercaptopropanol). By JOHN A. LUETSCHER, JR., and WARFIELD T. LONGCOPE, Baltimore, Md.

Thirty cases of arsenical intoxication have been treated with BAL. Dermatitis has been the commonest form of intoxication, and has responded to the inunction or injection of BAL. A few cases of hepatitis and of blood dyscrasias have been treated with less obvious benefit. No deaths occurred among these patients. The urinary excretion of arsenic has been followed in 17 patients receiving BAL. An increased excretion of arsenic

regularly followed BAL treatment of arsenical dermatitis, and reappeared on repeated courses of treatment. The increase of urinary arsenic coincided with the appearance in the urine of a substance with chemical characteristics resembling BAL. The arsenic excretion in hepatitis was not regularly affected by injections of BAL.

Forty-one cases of mercury poisoning have been treated with BAL. Two patients, treated 5 and 14 hours after poisoning, died. No deaths have occurred when BAL was given within 4 hours of ingestion of mercury, regardless of the amount of poison. In a control series, a dose of less than 1 gram of mercuric chloride caused no deaths, but there were 27 deaths among 86 patients who took 1 gram or more. There have been no deaths in a comparable group of 25 patients who took 1 gram or more of mercuric chloride but who were treated with full doses of BAL within 4 hours.

Combined Quinine-Plasmochin Treatment of Vivax Malaria: Effect of Relapse Rate. By HARRY MOST, CHARLES A. KANE, IRVING M. LONDON, and EDMUND F. SCHROEDER (by invitation), and PAUL H. LAVIETES, Swannanoa, N. C.

Quinine sulphate 1.0 gram and plasmochin naphthoate 0.02 gram simultaneously at 8-hour intervals for one day followed by quinine grams 0.65 and plasmochin naphthoate 0.02 simultaneously at 8-hour intervals for the next 13 consecutive days were administered to 72 white patients with acute attacks of vivax malaria of Pacific origin who were followed for at least 120 days after treatment.

The clinical relapse rate and total failure rate during 120 days observation was 4 per cent and 11.1 per cent respectively, following the above course of treatment. This is in sharp contrast to clinical relapse rates of 75 to 85 per cent and total failure rates of 85 to 90 per cent based on similar observations after treatment of more than 500 patients for acute attacks of vivax malaria of Pacific origin with quinine, quinacrine or other antimalarial drugs.

Combined quinine-plasmochine treatment resulted in apparent cure in 90 per cent of patients as judged by the occurrence of clinical relapse or appearance of parasites without fever or symptoms during a period of 120 days after treatment. Using the same criteria only 10 to 15 per cent of cures follow treatment with quinine, quinacrine or other antimalarial drugs currently in use.

No conspicuous or serious toxic manifestations were observed in 100 white patients who received combined quinine-plasmochin therapy for 14 consecutive days.

A Study of the Prophylactic Effect of Several 8-Amino Quinolines Including Plasmochin for South Pacific (Chesson) Vivax Malaria. By ALF S. ALVING and (by invitation) LILLIAN EICHELBERGER, BRANCH CRAIGE, JR., RALPH JONES, JR., THEODORE N. PULLMAN, and C. MERRILL WHORTON, Chicago, Ill.

James, in 1931, demonstrated that plasmochin in doses of 80 mgm. a day for 3 days, followed by 60 mgm. a day

for 5 days, starting the day before sporozoite inoculation, protected volunteers against *Plasmodium vivax* (Rumanian) infections. Feldman, *et al*, obtained only partial prophylaxis in *Plasmodium vivax* (McCoy) infections on the same regime.

In order to ascertain (1) whether plasmochin is an effective prophylactic drug against South Pacific (Chesson) strain of *Plasmodium vivax*, and (2) whether the prophylactic effect of plasmochin is a unique property of that drug or is shared by related compounds, four 8-amino quinolines, including plasmochin, were similarly tested on inmate volunteers at Stateville Penitentiary. Plasmochin was administered in daily doses of 90 mgm. (free base). The other drugs, SN–1452, SN–11,191, SN–13,276, were given at or near the maximum tolerated dose. Of 11 volunteers, 6 have been protected against infection by plasmochin, SN–1452, or SN–11,191 for 13 months. SN–13,276 has protected 9 out of 10 volunteers treated, the longest period of observation being 5 months. Several of the patients who were not protected were later cured by drugs not ordinarily curative in vivax infections.

Cure of Subacute Bacterial Endocarditis with Penicillin. By ARTHUR J. GEIGER and FRANCIS G. BLAKE, New Haven, Conn.

Of 20 patients treated in the past 2 years with penicillin in massive doses, cure was achieved in 19. One relapse and one re-infection were encountered in the ultimately cured cases. The underlying heart disease was congenital in 2, and rheumatic in the others. The patients ranged from 3 to 65 years in age, and none of the 17 survivors are invalids.

The penicillin resistances of the *Streptococcus viridans* ranged from 0.01 to 0.05 units per ml., and no increase in resistance developed in cases requiring more than one course of therapy.

Four methods of administration were tested: (1) fractionated intramuscular injections, (2) continuous intramuscular infusion, (3) continuous intravenous infusion, and (4) one or 2 daily intramuscular deposits of penicillin in oil and beeswax. Serum penicillin concentrations attained with each mode of treatment indicated the superiority of the continuous intravenous and the intramuscular depot (in oil and beeswax) methods for maintaining sustained penicillin effects.

Penicillin administered in the cured cases ranged from 3,900,000 to 102,000,000 units given for 3 weeks to 16 months.

In one cured case, which died 9 months later of another disease, the fibrocalcific and abacterial lesion of the previous endocarditis proved anatomically that the infection had been eradicated.

The Relationship of Serological Types of Group A Hemolytic Streptococci to Toxin Production and Antibody Response. By LOWELL A. RANTZ, San Francisco, Calif., WESLEY W. SPINK, Minneapolis, Minn., and (by invitation) PAUL J. BOISVERT.

A large number of Group A hemolytic streptococcal respiratory infections in young men were studied. The

etiological agents were classified into types by the precipitin technique of Lancefield.

When Dick positive individuals were infected by streptococci of types 17, 19, and 30, the acute illness was usually associated with a skin rash and skin sensitivity to the erythrogenic toxin was lost during convalescence. These phenomena were absent when the infectious agent was a strain of any other type except 3. In the latter instance the Dick test was usually reversed in the absence of demonstrable rash.

Close correlation has been discovered to exist between the amount of fibrinolysin formed by strains of several types *in vitro* and their ability to stimulate the production of antifibrinolysin in infected human beings.

The quantitative estimation of streptolysin production *in vitro* has been technically unsatisfactory but the magnitude of the antistreptolysin response is much greater following infection by certain types than by others.

These observations establish the fact that certain strains or types of Group A streptococci differed from one another in a constant manner in regard to important biological properties and in their effect on the human host.

Experimental Transmission of Minor Respiratory Illness to Human Volunteers. By THEODORE J. ABERNETHY, Ft. Bragg, N. C.

Attempts were made to induce respiratory disease in human volunteers by means of filtered secretions obtained from two single donors (OL and NE), both of whom had acute febrile respiratory illnesses without pneumonia. Immunity to re-inoculation with homologous and heterologous filtrate was also tested. Thirty-eight volunteers, kept in strict isolation before and after inoculation, comprised the study group.

Two clinically distinguishable types of minor illness, with different incubation periods, were induced. Thirteen of 19 volunteers receiving "OL" filtrate developed illnesses after 1 to 2 days; the principal clinical features were sneezing, nasal obstruction, coryza, and cough. Fourteen of 19 persons inoculated with "NE" filtrate became ill after an incubation period of approximately 5 to 6 days; sore throat was the outstanding feature and nasal symptoms were minimal. Partial immunity to the re-inoculation of homologous filtrate was found in the group which received "NE" inoculum; none was found in the subjects given "OL" filtrate. Cross immunity was not demonstrated with either filtrate. Atypical pneumonia was not induced. Controls inoculated with their own filtered respiratory tract secretions remained free of symptoms.

The results indicate that at least two filtrable agents, presumably viruses, can induce minor respiratory illness in man.

Studies of the Incidence and Pathogenicity of Pleuropneumonia-like Organisms in Humans. By LOUIS DIENES and WILLIAM E. SMITH (introduced by MARIAN W. ROPES), Boston, Mass.

Pleuropneumonia organisms are important pathogens in animals, producing chronic diseases usually with in-

volvement of joints. Pathogenicity in humans is suggested by the present studies.

Organisms of the pleuropneumonia group (L organisms) were first cultured from the human genitourinary tract in 1937. Their incidence in the female genitourinary tract is relatively high, 58 out of 244, (26 per cent). In males, the incidence is only 6 out of 71 (8 per cent) and L organisms have been found in only 24 patients. In females, these organisms have been found predominating or in pure culture in acute inflammatory and suppurative processes of the genito-urinary tract. In males, all of the 24 patients with positive cultures had prostatitis. The organisms were obtained in pure culture in 5 cases and disappeared after the prostatitis subsided in 3 cases. There was a severe cystitis in one patient and in one a periurethral abscess from which pure cultures of L organisms were obtained.

A relation of L organisms to an infectious type of arthritis is suggested by the fact that 12 of the 24 male patients had acute joint involvement at the time the prostatic cultures were positive. Four presented the characteristics of Reiter's disease with urethritis, arthritis and purulent conjunctivitis. In one of these cases L organisms were cultured from the prostate during 2 attacks 1½ years apart. The second of these attacks followed a prostatic massage, the secretion from which was negative for L organisms. Two weeks later, urethral discharge showed an abundant growth of L organisms in pure culture. In 2 patients L organisms were cultured from synovial fluid. Acute joint involvement was rare in women, being observed in only 4. The husband of one had a positive prostatic culture, and both developed acute arthritis within a few weeks after marriage.

Sulfonamides and penicillin did not affect the genitourinary or joint symptoms. The results of treatment with streptomycin were sufficiently suggestive to warrant further trial.

These observations suggest that pleuropneumonia-like organisms have pathogenic activity in the male and female genitourinary tracts, and may be related to an acute infectious type of arthritis.

The Site of Origin of "Blackout" in Aviators. By E. H. LAMBERT (introduced by C. F. Code), Rochester, Minn.

Blackout is a temporary loss of vision without loss of consciousness which aviators experience when subjected to centrifugal force in aircraft or on a human centrifuge. In the investigations to be reported, temporary loss of vision was produced at 1 g. (gravity) by application of air pressure to the eyeball. When the effective systolic arterial pressure at the eye (systolic pressure at head level minus the pressure applied to the eye) was 49 to 30 mm. Hg vision was dimmed; peripheral vision was lost at 32 to 20 and vision was completely lost at 21 to 0. These visual changes had the same latent period, progress of development and level of effective blood pressure as the visual changes produced by centrifugal force. Application of 20 to 30 mm. Hg pressure to the eyeball when the subject is exposed to centrifugal force lowers the threshold

of force at which visual changes occur by 1 g. Suction of 30 to 40 mm. Hg applied to the eyeball prevents the occurrence of blackout when the man is exposed to centrifugal force. These experiments allow the conclusion that blackout is of retinal origin.

Some Effect of Injected Cytochrome C on Myocardial and Cerebral Anoxia in Man. By SAMUEL PROGER and (by invitation) DEMETRE DEKANEAS, Boston, Mass.

1. The effects of anoxia on the electrocardiogram can be prevented by the injection of cytochrome C.

2. Injected cytochrome C has an equivocal effect in prolonging exercise tolerance in patients with angina pectoris. It has no noticeable immediate effect on myocardial infarction.

3. Subjects seem to tolerate anoxia more easily when they have been previously injected with cytochrome C.

4. The effects of anoxia on the electroencephalogram can be largely prevented by the intravenous injection of cytochrome C.

5. The effects of anoxia in impairing visual discrimination can be overcome by the intravenous injection of cytochrome C.

6. The effects of anoxia in slowing the cerebral functions required for code transliteration can be overcome by the injection of cytochrome C.

Acclimatization to Intermittent Anoxia. By WRIGHT ADAMS, Chicago, Ill.

Alveolar carbon dioxide and oxygen tensions have been measured in fifteen male subjects during sixteen series of exposure to a simulated altitude of 10,000 feet above sea level in a decompression chamber. The subjects spent five hours daily for six days of each week for six consecutive weeks in this mildly anoxic condition. The alveolar samples were taken more than three hours after a meal and after more than three hours of continuous exposure to simulated altitude.

The carbon dioxide tension, after an initial reduction, showed a definite tendency to drop progressively during the first weeks of intermittent exposure. The mean tension during the preliminary control period was 40.0 mm. Hg. During the first week of exposure it was 36.9 mm., during the second and third weeks it was 35.8 mm., and during the last three weeks 35.1 mm. There was considerable individual variation in both the initial reduction and the progressive decrease with prolonged exposure.

The oxygen tension, after an initial reduction, tended to increase during the course of intermittent exposure. The increase was greatest in those subjects who showed the greatest progressive decrease of carbon dioxide tension, but the oxygen values were more variable from day to day.

Acclimatization to Humid Heat: A Function of Adrenal Cortical Activity. By JEROME W. CONN and (by invitation) MARGARET W. JOHNSTON and LAWRENCE H. LOUIS, Ann Arbor, Mich.

Our studies indicate that the process of acclimatization to heat is characterized metabolically by (*1*) negative

nitrogen balance, independent of the composition of the diet, and (2) sharply falling concentrations of sodium and chloride in sweat and urine. Similar findings have been observed in normal animals treated with large doses of adrenal cortical extract.

If, during acclimatization, the negative N balance represents a secondary expression of increased adrenal cortical activity, the primary stimulus for which the need is to conserve salt, the administration of desoxycorticosterone acetate (D.C.A.) should serve as a useful tool in dissecting the mechanism since (1) it could remove the pressure on the adrenals for the production of a substance capable of retarding body losses of salt, and (2) *per se*, it has no significant effect upon protein metabolism.

D.C.A. was administered to men (1) unacclimatized to heat and living in a temperate climate, (2) undergoing acclimatization to heat, and (3) fully acclimatized to heat. Results:

1. It always diminishes markedly the concentration of sodium and chloride in sweat and urine, the most persistent effect being upon sweat.

2. During acclimatization (with an existent negative N balance) it results in a sharp approach to nitrogen equilibrium.

3. *Upon cessation of D.C.A.* a very marked rise in the concentration of sweat salt always occurs. The process of acclimatization is temporarily impeded, and in fully acclimatized men acclimatization is temporarily lost.

Conclusions: 1. Increased adrenal cortical function is importantly involved in the process of acclimatization to heat.

2. Correction by exogenous D.C.A. of the negative N balance characteristic of the process of acclimatization indicates that production of endogenous adrenal cortical substance (which affects both electrolyte and nitrogen metabolism) has been diminished; and that the original stimulus to the adrenals (under these conditions) represents the need to conserve body salt.

3. Temporary loss of acclimatization and loss of the ability to produce sweat dilute in sodium chloride upon withdrawal of D.C.A. suggests that it requires several days for the adrenal cortices to again reach a high degree of functional activity, after having been put at relative functional rest by a few days of aid from an exogenous source (D.C.A.).

Oxygen Content of Pulmonary "Capillary" Blood in Unanesthetized Human Beings. By L. DEXTER, C. S. BURWELL, and (by invitation) F. W. HAYNES, and R. E. SEIBEL, Boston, Mass.

The venous catheter technique of Cournand and Ranges (1941) has been used in the present study. A 9F venous catheter, 100 cm. long, with a single hole directly on the tip, has been introduced through the median basilic vein into the right auricle, right ventricle, and pulmonary artery, usually the right branch. The catheter has then been pushed as far as possible into a small branch of the pulmonary artery and wedged so as to obstruct this branch. Dog experiments have indicated no deleterious

effects physiologically or pathologically from this procedure providing the catheter does not plug the pulmonary arterial branch for more than one hour. With the catheter in place, blood samples have been withdrawn under oil and their oxygen content and saturation compared with those of blood withdrawn from the femoral artery. In a series of normal individuals, the oxygen saturation of blood obtained from the pulmonary artery by this method has been found to be 95 to 98 per cent, corresponding closely to the oxygen saturation of the femoral arterial samples. Samples from other parts of the pulmonary artery have uniformly been less oxygenated as would be expected of mixed venous blood. That the blood so obtained represents blood which has flowed from pulmonary capillaries and possibly pulmonary veins in a retrograde fashion from the pulmonary bed distal to the tip of the catheter rather than from systemic arteries emptying into the pulmonary artery is borne out by the fact that in a group of cyanotic patients with congenital heart disease and a right-to-left shunt, blood from the femoral artery has had varying degrees of oxygen unsaturation, while blood obtained from the distal portion of the pulmonary artery as described has been almost fully saturated with oxygen.

This procedure appears of value in differentiating cyanosis of pulmonary origin from that due to a venous shunt, and in determining the oxygen content of blood entering the left auricle.

Demonstration that the Human Right Ventricle Obeys Starling's Law during Physiological Changes in Respiration. By HENRY D. LAUSON, ANDRE COURNAND, and RICHARD A. BLOOMFIELD (introduced by Dickinson W. Richards, Jr.), New York, N. Y.

Although Starling's law of the heart is widely accepted, its operation in man still requires further proof.

Simultaneous right ventricular and intrapleural pressures were recorded during quiet and forced respiration in three recumbent tuberculous patients, with recent pneumothorax, whose circulation was considered essentially normal. Pressures in successive cardiac cycles were measured (a) exactly at the end of diastole, and (b) at the peak of the following systole. The concurrent intrapleural pressures were subtracted to give the "net initial" and "net systolic" pressures, which are related to the degree of stretch of the filled ventricle, and to the degree of response to that stretch, respectively.

Analysis of several hundred cardiac cycles revealed (a) an inverse correlation between the intrapleural pressure and the two net ventricular pressures; and (b) a direct linear relationship between the net initial pressure and the net systolic pressure of the same beat. For example, a net initial pressure of 2 mm. Hg was followed by a net systolic pressure of 22 mm. Hg during expiration, while during inspiration the former increased to 8 mm. Hg and the latter increased to 36 mm. Hg.

The results constitute a direct demonstration of the essential features of Starling's law in man under physiological conditions.

The Role of the Kidney in Chronic Heart Failure: Evidence of a Forward Failure Hypothesis of Edema Formation. By A. J. MERRILL (introduced by E. A. Stead, Jr.), Atlanta, Ga.

The cause of edema in chronic cardiac failure has never been established. Everyone agrees that the fluid intake must exceed the fluid output and that the edema fluid accumulates because of failure of the kidney to excrete the excess salt and water. The question of why the salt and water is retained has not been answered. The proponents of the backward failure theory believe either that it is forced into the tissues by a high venous pressure or that it is retained by the kidney because of the adverse effect of a high venous pressure on kidney function. The observations recorded here are not in agreement with this concept, but point to a marked decrease in renal blood flow from forward failure as the primary mechanism leading to edema formation.

Patients with chronic failure who formed edema at rest were studied. The renal blood flow was uniformly reduced to one-third to one-fifth of normal. The filtration rate was reduced to one-half to one-third of normal. Thus the amount of sodium presented to the tubules was greatly reduced and tubular reabsorption of the sodium was nearly complete, though the absolute amount absorbed was less than in the normal.

The reduction in renal blood flow to this extreme level was unrelated to the level of the venous pressure. It could be correlated with the level of the cardiac output.

The kidney at rest normally received from one-fourth to one-fifth of the cardiac output. This large blood flow is not necessary to maintain the life of the renal cells. It seems to be important in the normal metabolic functions of the kidney and in urine formation. When the cardiac output becomes inadequate for the needs of the body, vasoconstriction in the kidneys allows shunting of blood elsewhere without permanent injury to the kidneys. It is believed that this fall in renal blood flow is the primary cause of edema in patients who have chronic failure and who become edematous at rest.

Metabolic Changes in Young People with Coronary Heart Disease. By JACOB LERMAN and PAUL D. WHITE, Boston, Mass.

This study was stimulated by 2 types of observations: (*1*) the clinical study of young persons with coronary heart disease by Glendy, White and Levine; (*2*) though myxedematous patients with angina often get worse on thyroid, they may, if thyroid is administered cautiously, actually improve or get rid of their angina. This led to observing the effect of thyroid in coronary disease and the rôle of metabolic disturbances in its pathogenesis. It was deemed advisable to limit the study to young people (40 years and under).

We have data on 28 patients, 25 of them being males. The most striking finding has been the relative frequency of low metabolic rates and of high cholesterol levels in the blood. Adequate BMR levels, ranging between plus 3 and minus 36, were obtained in 27, and in 21 they were under minus 10. The blood cholesterol ranged between 179 and 480 mgm. per cent, the majority (22 out of 28) being over 250 mgm. per cent. Blood sugar and serum protein values were within normal limits. There were no stigmata of endocrine disturbance in any case.

On thyroid, the BMR levels tended to rise and the blood cholesterol to fall. Although it is too early to determine the effectiveness of thyroid, results thus far indicate that in all cases with angina but 2, the administration of thyroid in small dosage was associated with diminution or disappearance of pain; in cases without angina, it did not precipitate angina or any other untoward symptom.

Tissue Circulation. Pulmonary Ventilation as Measured by Gas Exchange with a Note Regarding Decompression Sickness and Polycythemia Vera. By H. B. JONES (by invitation) and J. H. LAWRENCE and J. G. HAMILTON, Berkeley, Calif.

During the inhalation of pure oxygen the nitrogen is washed out of the lungs and removed from the tissues. The nitrogen elimination can be determined by analysis of inert gas residues in expired gases. The data can be fractionated into portions coming from lung gases and various body tissues. The lung components give a measure of pulmonary ventilation efficiency. Eleven of twelve polycythemias studied showed a reduction of portions of their pulmonary exchange. Such an effect was only noted in two subjects of a normal series of forty-three.

As determined by radioactive inert gases and nitrogen exchange, the rate of nitrogen elimination from the body tissues has been found to be a measure of the perfusion of blood through the tissues. The body can be divided into two to four groups of tissues on a basis of volume of tissue and blood supply from gas exchange measurements. The tissue vascularity in the twelve polycythemia patients studied was within the normal range.

The gas exchange rate can be shown to be a precise indicator of prediction of decompression sickness and degree of protection from decompression sickness by nitrogen elimination during oxygen inhalation, for group measurements.

The Effects in Man of Blockade of the Autonomic Ganglia by Tetraethyl Ammonium Bromide. By RICHARD H. LYONS and (by invitation) GORDON K. MOE, ROSALIE B. NELIGH, SIBLEY W. HOOBLER, KENNETH N. CAMPBELL, ROBERT L. BERRY, and B. R. RENNICK, Ann Arbor, Mich.

The parenteral administration of tetraethyl ammonium bromide in animals produces blockade of autonomic ganglia as judged by ganglionic paralysis of the nictitating membrane, functional denervation of the heart, and a decrease in blood pressure with increase in peripheral blood flow.

The effects of this drug in man indicate that it may be useful in several ways. It produces a vasodilatation in the extremities, as measured by changes in skin tempera-

ture, of the same degree as that found under spinal anesthesia or sympathectomy. There is striking relief of pain in vasospastic peripheral vascular disease and in causalgic states. It produces a significant decrease in both systolic and diastolic pressure in many patients with hypertension. Symptoms of hypertensive encephalopathy and of dyspnea with hypertensive heart failure have been temporarily relieved in the few cases so treated. The decrease in diastolic pressure with test doses of the drug tends to approximate the diastolic pressure found two weeks after splanchnic section in hypertensive patients.

Other evidence of blockade of the autonomic ganglia resulting from the drug is the development of severe postural hypotension in hypertensive patients as well as in normal subjects, loss of sweating, cessation of gastrointestinal motility, increase in heart rate, decrease in venous pressure, dry mouth, mydriasis with loss of accommodation and ptosis.

The cardiac output as measured by the ballistocardiogram is increased about 20 per cent in normal and hypertensive subjects. The renal blood flow remained fairly constant in spite of the drop in blood pressure though there was a decrease in filtration rate proportionate to the fall in blood pressure.

The drug is rapidly excreted by the kidneys when given either intravenously or intramuscularly so that some of the effects are usually of relatively short duration.

The Effects of Bed Rest and Immobilization upon some Aspects of Calcium Metabolism and Circulation in Normal Men: Their Modification by the Use of the Oscillating Bed. By JOHN E. DEITRICK and G. DONALD WHEDON (introduced by Ephraim Shorr), New York, N. Y.

Four normal men were studied on a metabolism ward during control (6 to 8 weeks) bed rest (6 to 7 weeks), and recovery (4 to 6 weeks) periods. Immobilization was standardized by plaster casts from umbilicus to toes. Diets were constant.

Total calcium losses ranged from 8.95 to 23.9 grams, maximum daily loss reached at the fifth to sixth week. Urinary calcium excretion during bed rest more than doubled in all four men. Despite this, no compensatory mechanism for increasing calcium solubility in the urine was demonstrated. pH of urine rose slightly. Citric acid excretion and urine volumes remained relatively constant.

Deterioration of postural circulatory mechanisms was the principal effect upon the circulation produced by bed rest. Subjects showed an increased tendency to faint on tilting to 65°. Experiments suggest that the most important factor in this deterioration is a decrease in venous return of blood from the legs.

Oscillating Bed: The experiment was repeated on two of the original subjects under identical conditions except for the use of a motor-driven oscillating bed eight hours a day during immobilization. Total calcium losses were reduced by 50 to 70 per cent from that occurring in the fixed bed. Urinary calcium increased only one-third to one-half as much. The oscillating bed tended to prevent

the deterioration of circulatory control in the upright position.

Studies on Chronic Thyrotoxic Myopathy. By GEORGE W. THORN, and (by invitation) HOWARD A. EDER, Boston, Mass.

In addition to the generalized weakness accompanying thyrotoxicosis, several forms of myopathy have been reported. In some patients the muscular weakness and atrophy are profound and often out of proportion to the degree of thyrotoxicosis. This syndrome has been termed chronic thyrotoxic myopathy. During the past two years we have studied five such patients. The disease is characterized by symmetrical muscular weakness and atrophy which involves the muscles of the shoulder and pelvic girdle particularly. All patients showed a striking disturbance in creatine metabolism and elevated basal metabolic rates ranging from plus 33 to plus 60 per cent. One patient died before adequate treatment could be instituted. Four patients have shown striking and continued improvement following treatment of thyrotoxicosis by iodine plus subtotal thyroidectomy or thiouracil. Possible mechanisms whereby certain patients with thyrotoxicosis develop profound muscular atrophy is described.

Two patients with myasthenia gravis complicated by thyrotoxicosis have also been studied. Striking improvement in the myasthenia gravis was observed following treatment of the *thyrotoxicosis.* The remarkable improvement which may occur in the clinical condition of patients suffering from myopathy complicated by thyrotoxicosis indicates the importance of establishing the diagnosis and instituting appropriate therapy.

Evidence that Chorionic Gonadotrophic Hormone Stimulates Human Leydig-Cell Function and Maturation; and that Purified Follicle-Stimulating Hormone Stimulates Spermatogenesis. By CARL G. HELLER and WARREN O. NELSON (introduced by Edwin E. Osgood), Portland, Ore. and Iowa City, Iowa.

Biopsies of the testes of twenty adult prepuberal eunuchoids proved that they had Leydig-cells which were undeveloped and seminiferous tubules which failed to carry on active spermatogenesis. Assays of urinary gonadotrophins revealed depression of follicle-stimulating hormone (F. S. H.) and interstitial-cell-stimulating hormone (I. C. S. H.).

Administration of chorionic gonadotrophins, 750 I. U. twice daily intramuscularly, promptly resulted in stimulating the production of androgenic hormones, as judged by the development of the secondary sex characteristics. Estrogen and 17-ketosteroid excretion rose concomitantly adding further evidence of androgenic stimulation. Testicular biopsies taken 3 to 6 months later revealed that the interstitial cells of Leydig had developed normally and were in a secretory state. Thus one can conclude that the chorionic gonadotrophins specifically stimulate the morphological maturation of the interstitial cells of Leydig and apparently stimulate them to secrete androgen.

The seminiferous tubules increased in size but spermatogenesis was not initiated. Therefore a purified follicle-stimulating hormone (F. S. H.) (prepared by enzymatic digestion by Dr. Hartford McShan of the University of Wisconsin) was injected daily for several months to a number of the patients, while continuing the chorionic gonadotrophic therapy. This resulted in active spermatogenesis as revealed by examination of seminal fluid and testicular biopsies.

Measurement of an Insulin Antagonist in the Serum of an Insulin Resistant Patient by the Blood Sugar Curve Method in Mice. By FRANCIS C. LOWELL, Boston, Mass.

Serum from a patient resistant to insulin had previously been shown to prevent hypoglycemic convulsions in mice injected with insulin due, apparently, to the presence in the serum of an antibody for insulin. The use of the blood sugar curve method in mice injected with mixtures of serum and insulin has made possible the demonstration of insulin neutralization with much smaller amounts of serum than heretofore. This has permitted repeated tests on a single blood specimen and the insulin-neutralizing activity could be measured with a certain amount of accuracy. Serum from the patients and from normal individuals were studied. A total of 25 tests were done, 6 animals were used in each and three blood sugar determinations, at 30 minute intervals, were made in each animal.

In addition to the clear-cut demonstration of insulin-neutralization by the patient's serum, the results indicated that more than 1,000 units of insulin, given in a short period of time, would have been required to control the patient's resistance to insulin.

It is concluded that an immune mechanism such as that which probably was present in the patient studied, will readily explain the extreme degree of insulin resistance which is occasionally encountered.

READ BY TITLE

The Thyrotropic Hormone: Its Inactivation by Elemental Iodine and Its Reactivation by Thiouracil and other Goiter Producing Compounds. By ALEXANDER ALBERT (by invitation) and RULON W. RAWSON, Boston, Mass.

The addition of elemental iodine to a solution of thyrotropic hormone precipitates the hormone as a brown iodoprotein complex which has no thyrotropic activity as measured by thyroid weight, loss of thyroid iodine and thyroid mean acinar cell height in the day-old cockerel. A direct relationship has been demonstrated between the amount of iodine added and the degree of inactivation of hormone. Mixing thyrotropic hormone with iodine as potassium iodide does not cause any loss of hormonal activity. The inactivation is not due to the involuting action of iodine on the thyroid.

The brown precipitate dissolves when treated with reducing agents, *i.e.*, *thiouracil*, potassium thiocyanate, amino mercapto thiadiazole and phenylaminomethyl mercaptothiazoline, ascorbic acid and sodium thiosulfate. Precipitates treated with the first four agents, which are goitrogenic, recover in varying degrees their original thyroid stimulating activity. The latter two reducing agents which are not goitrogenic did not materially reactivate the hormone. The goitrogenic agents in the amounts used to reactivate the hormone produced no change in the thyroid when administered alone, but augmented the thyroid stimulating effect when mixed with active hormone.

The Reaction of Hemophilic Blood to the Clot Promoting Factor in Normal Plasma and a Method for Its Quantitative Measurement. By BENJAMIN ALEXANDER and GRETA LANDWEHR (introduced by H. L. Blumgart), Boston, Mass.

A method has been devised for the quantitative measurement of the reaction of hemophilic blood to the clot promoting effect of normal plasma. This method is of value in estimating the coagulation defect found in this disease.

Minute amounts (0.00005 cc.) of normal plasma hastened the coagulation of hemophilic blood *in vitro*, and .002 cc. lowered the clotting time to normal. A logarithmic plot of added plasma against the coagulation time was linear in two hemophiliacs. The curves had different slopes. In one patient the effect could be represented by the equation:

$$0.29\text{–}2.7 \text{ log (clotting time}_{min}.) = \text{log normal plasma}_{cc}.$$

This effect was reproducible and accurately predictable over 5 months' observation.

Fresh or freshly processed frozen plasma from different individuals had the same potency.

The infusion of 150–200 cc. of fresh or freshly processed plasma lowers the clotting time of the blood of two hemophiliacs to normal; coagulation remains normal for about 24 hours, and thereafter slowly returns to its previous level within 3 to 4 days. Two cases treated 3 times weekly with this amount of plasma over 5 months have never failed to respond. The coagulation times were maintained at decidedly lower levels.

By this approach, control of the abnormal coagulation in hemophilia is feasible.

Penicillin Aerosol with Negative Pressure in the Treatment of Sinusitis. By COLTER RULE and BETTINA GARTHWAITE (introduced by Alvan L. Barach), New York, N. Y.

When oxygen is passed through a nebulizer containing a solution of 40,000 to 50,000 units per ml., the nebulin (or aerosol) produced may be delivered to the nasal and oral pharynx as well as to the bronchial and alveolar surface. A valve has been constructed by which intermittent suction develops a negative pressure (45 to 60 mm. Hg) in the antrum. After treatment penicillin has been recovered from antral washings. Acute and chronic cases of sinusi-

tis have been treated with clearing of symptoms and radiological evidence of disease.

Lantern slides will be used to show the effect of this procedure on purulent sinusitis and also illustrative cases of chronic bronchitis and bronchiectasis in which inhalation of penicillin aerosol was used.

Nitrite Protection against Cyanide Poisoning. By ALLAN D. BASS (by invitation) and WILLIAM T. SALTER, New Haven, Conn.

It was demonstrated by Chen, Rose and Clowes that nitrites, with thiosulfate, afford a definite protection against injected cyanide. The mechanism involves the formation of methemoglobin in the circulating erythrocytes. This work has been extended to hydrocyanic acid in gaseous form. A nomogram has been constructed to show the limiting concentrations and time intervals in terms of blood methemoglobin concentration.

Dogs, maximally protected, show no obvious symptoms for over an hour in an atmosphere so concentrated in cyanide that the control (unprotected) animal succumbs within two minutes. When protected dogs finally lose consciousness, they recover much more rapidly when rescued than do unprotected animals. Indeed, the latter frequently fail to recover.

This "antidote" is one of the few known which have a rational mechanistic basis after systemic absorption. Certain applications to civilian hazards are possible within the limitations imposed by methemoglobinemia and hypotension.

This work was released with the approval of the OSRD Committee on Medical Research, under whose auspices the observations were made.

A Critique of Physical Fitness Tests. By WILLIAM B. BEAN and (by invitation) C. R. PARK and D. M. BELL, Ft. Knox, Ky.

The Army needed tests to assess soldiers' fitness for active duty; to evaluate convalescence, to compare different units and to measure the effects of training. Since there was no general agreement on the meaning of the term fitness, or the role of physique, physiologic state and psychologic factors, several types of performance test were employed extensively. The Harvard Fatigue Laboratory Step Test, the Army Air Forces Test and the Army Ground Forces Test were run 6 times in 8 weeks by approximately 700 soldiers during controlled training in a field trial of combat rations. All tests suffered from failure to control motivation, from defective scoring systems and from the influence of learning on scores. There was poor agreement between different tests. No test considered the post-exercise state and only one evaluated physiologic cost. In the group studies no correlation occurred between individual scores and incidence of minor clinical and biochemical abnormalities. It was concluded that there is no abstract state of fitness—its definition must include a particular task and tests to measure it should resemble that task. On the basis of wartime experience a program of fundamental research is needed to define fitness better and devise more satisfactory tests.

Studies on Immunity to Bacterial Pyrogens. By PAUL B. BEESON, Atlanta, Ga.

The present work is part of an investigation of the mechanism by which patients undergoing typhoid vaccine fever therapy develop a marked tolerance to this pyrogenic stimulus. Rabbits were given daily injections of the same dose of vaccine, and were found to exhibit a progressive diminution in febrile response during the first 6 to 10 injections, after which they reacted to each injection with low-grade fever of approximately the same extent. Rabbits which were given increasing quantities of bacterial vaccine were able to withstand intravenous inoculation of large doses of vaccine with only slight fever. The same doses caused hyperpyrexia and death in normal control animals. Animals rendered immune to the effects of typhoid vaccine were tested with vaccines of heterologous bacterial species (Ps. aeruginosa and S. marcescens), and were found to be similarly immune to them. The same type of immunity was induced with purified typhoid bacterial pyrogen, a carbohydrate substance.

A striking effect was produced in pyrogen-immune animals by reticulo-endothelial blockade, using either Thorotrast or trypan blue. This completely abolished the immunity, so that the animals responded to the injection of the same dose of vaccine with prolonged, high fevers.

The immunity to bacterial pyrogens has several peculiar features. It is maximal on the day following the last injection of pyrogen and disappears completely in from 1 to 3 weeks. There is apparently no relationship to the production of specific antibodies, since the immunity applies to heterologous bacterial species and since it can be provoked by the purified pyrogen, which is not antigenic. Furthermore, the immunity cannot be passively transferred with serum. Elevation of body temperature plays no part in the development of the immunity, since prevention of fever by administration of amidopyrin during the period of training did not interfere with the development of immunity. Also, animals given a series of mechanically-induced fevers did not develop immunity to bacterial pyrogens. In view of the effect of reticulo-endothelial blockade, it would appear that the development of immunity to bacterial pyrogens involves an alteration in the functional activity of the reticulo-endothelial system.

The Folic Acid Content of Leukocytes: Observations on Normal Subjects and Persons with Leukemia. By FRANK H. BETHELL and (by invitation) MARION E. SWENDSEID, Ann Arbor, Mich.

Folic acid has been shown to be an essential factor for normal hematopoiesis in several species of animals, and the recent observations of Spies and others indicate that this substance may be required for orderly erythropoiesis in humans. In earlier studies, on the rat, it has been shown that a deficiency of folic acid is followed by arrested maturation of the precursors of all of the formed elements of the blood, resulting in progressive leukopenia, anemia

and thrombocytopenia. These observations suggest that altered metabolism of folic acid may play a role in hematopoietic disorders characterized by excessive or disorderly proliferation of blood cells or their precursors. Accordingly, determinations have been made of the content of folic acid in the leukocytes of healthy subjects and in those of persons with acute and chronic forms of leukemia.

In twelve normal subjects the content of folic acid varied from 40 to 180 micrograms per cubic centimeter of packed white cells with an average value of 80. In six cases of chronic lymphogenous leukemia the range of value was 70 to 160 micrograms, with a mean of 108. In seven cases of chronic myelogenous leukemia a range was obtained of 75 to 220 micrograms, with a mean of 146. In persons with chronic leukemia the highest folic acid values were found in association with the greatest percentages of relatively immature white cell elements. Determinations made after irradiation therapy in four cases of chronic leukemia demonstrated a decrease in leukocyte folic acid content which coincided with a reduction in the percentage of the more immature cellular elements.

In seven cases of acute leukemia, six of myeloblastic and one of leukosarcoma, the range of values for folic acid was from 250 to 800 micrograms per cubic centimeter of white cells with an average of 460. This value is five times that of the mean folic acid content of the leukocytes of normal subjects. Whether excessively large amounts of folic acid are required by the younger and more rapidly growing leukemic cells or whether the high content of the substance in such cells represents a failure of utilization or metabolic dysfunction is not answered by these observations. However, it may be concluded that, in leukemia, a direct relationship exists between the amount of folic acid contained in the white cells of the peripheral blood and the degree of immaturity of the cells.

Foreign Body Emboli to the Heart and Lungs. By EDWARD F. BLAND, Boston, Mass.

In connection with a comprehensive survey of wounds of the heart in the Mediterranean Theater, four unusual cases were encountered in which large shell fragments migrated centrally by way of the blood stream: in three instances to the lungs, and in one to the right ventricle. Each patient presented features of special interest:

Case 1. Autopsy revealed penetration of the inferior vena cava and a large 10.5 gm. metal slug (2.5 × 1.0 × 1.0 cm.) in the chamber of the right ventricle. Death on the 13th day was due to multiple pulmonary emboli.

Case 2. Autopsy revealed penetration of the inferior vena cava and an embolic shell fragment (1.5 × 0.6 × 0.4 cm.) in inferior branch of the left pulmonary artery. Death on the 14th day resulted from intraperitoneal hemorrhage.

Case 3. Thoracotomy 6 weeks after a cervical wound revealed a metallic shell fragment in the inferior branch

of the right pulmonary artery. It was not removed and recovery has been complete.

Case 4. X-ray showed a large metal fragment in the left hilar region; not found on surgical exploration. Post-operative X-ray showed the foreign body now in the right hilar region; surgical exploration revealed the fragment impacted in the right main pulmonary artery. It was not removed. Recovery has been complete with only slight dyspnea on considerable effort.

It is noteworthy that in the three instances where lodgement occurred in a large pulmonary artery neither pulmonary infarction nor significant symptoms ensued. A shift of the large metal fragment from the left main pulmonary trunk to the right main trunk in Case 4 is remarkable.

Increased Protein Content of the Cerebrospinal Fluid in Rheumatoid Spondylitis. By EDWARD W. BOLAND and NATHAN E. HEADLEY (by invitation) and PHILIP S. HENCH, Rochester, Minn.

Fifty soldiers were studied, 33 with rheumatoid spondylitis alone, 17 with rheumatoid spondylitis and arthritis of peripheral joints. The total protein content of spinal fluid from the lumbar region was increased (between 46 and 105 mgm. per 100 ml.) in 42 per cent of the 50 cases. It is increased sometimes in rheumatoid arthritis of peripheral joints, more often in rheumatoid spondylitis alone, most often in rheumatoid arthritis of both spine and peripheral joints. The increase was related to the severity of the disease, but not to its duration or spinal spread. Colloidal gold reactions were generally normal.

The increase in spinal fluid protein in rheumatoid spondylitis is of about the same order as that in most cases of ruptured intervertebral disks. If the protein in any given case is increased notably above 100 mgm. per 100 ml., some cause other than spondylitis should be sought, even though spondylitis is present.

In peripheral rheumatoid arthritis excess protein may invade the subarachnoid space via the choroid plexus sometimes from increased plasma proteins, sometimes from an increased permeability of the choroid plexus when plasma proteins are normal. In rheumatoid spondylitis the same may occur but additional protein may enter the lumbar subarachnoid space via spinal perivascular and perineural spaces.

Studies of Hepatic Blood Flow in Man. By STANLEY E. BRADLEY (introduced by Chester S. Keefer), Boston, Mass.

The effects of increased intra-abdominal pressure and of drugs upon the hepatic blood flow in man were studied by means of a method previously described, which is based upon the hepatic clearance of Bromsulphalein.

1. Compression of the abdomen by a girdle pressurized at 80 mm. Hg was followed, in five of eight studies, by a rise in the concentration of BSP in the peripheral blood and a fall in the estimated hepatic blood flow (EHBF). On two occasions, the hepatic venous BSP

concentrations decreased, but on all others an elevation was observed.

2. Epinephrine, 0.4 to 1.0 ml. intramuscularly, in six of seven studies, was followed by increases in the concentration of BSP in the peripheral and hepatic venous blood. EHFB always increased, occasionally as much as three hundred per cent.

3. Histamine, 0.36 to 0.60 mgm. intramuscularly, caused very little change in four studies. A transient effect similar to that caused by the epinephrine was twice observed.

4. Glucose plasma levels above 500 mgm. per cent had no effect upon EHFB in three studies.

Errors Encountered in the Use of the Goldberger Central Terminal. By J. MARION BRYANT (by invitation) and FRANKLIN D. JOHNSTON, Ann Arbor, Mich.

This study was undertaken to determine whether electrocardiograms obtained with the central terminal of Goldberger, where equal large resistances between the extremity electrodes and the terminal are omitted, differ significantly from electrocardiograms obtained with the Wilson central terminal containing the 5000 ohm resistances.

In a series of 500 consecutive routine electrocardiographic examinations the left arm potential (V_L) was obtained using both methods. This lead was recorded first with 5000 ohm resistances between both the right arm and left leg and the central terminal, and then without disturbing the limb electrodes the right arm and left leg electrodes were short circuited and a second curve recorded. A significant difference was found between the curves obtained with the two methods in approximately 10 per cent of the individuals examined. The most common variation was a difference in the amplitude of the R deflection ranging from 2 to 7 mm. in 47 individuals.

These observations indicate that omitting the resistances from the central terminal as recommended by Goldberger produces significant differences in a small percentage of cases.

Rate of Water Loss from the Skin of Normal and Trench Foot Subjects. By G. E. BURCH and (by invitation) H. L. MYERS, R. R. PORTER, and N. SCHAFFER, New Orleans, La.

The rate of water loss (insensible and sensible perspiration) was measured simultaneously and quantitatively from the skin of the plantar and dorsal surfaces of the feet of 25 normal young male adults and 25 patients with mild chronic trench foot. The method used to determine the rates of water loss have been described (Neumann, Cohn and Burch; Burch and Sodeman). The subjects rested comfortably in bed in a comfortable room atmosphere and then in a hot and humid atmosphere.

There was no difference in the rates of water loss from the skin of the plantar and dorsal surfaces of the feet of normal subjects and patients with mild chronic trench foot, thus indicating that the skin showed a normal functional state and ability to inhibit water loss by diffusion,

and the sweat glands a normal functional ability to secrete sweat. Under comfortable environmental conditions, the plantar skin showed a greater rate of water loss than skin from the dorsum of the foot. A hot and humid atmosphere resulted in a much greater rate of sweating from the skin of the dorsal surface than that of the plantar surface, thus indicating the failure of the plantar skin to be concerned with emergency sweating for the purpose of heat loss. These findings are also probably of importance in the evolutionary development and preservation of man.

Measurements of the rate of water loss from the skin of the foot cannot be employed to detect a return to normal of relatively mild chronic trench foot.

The Significance of Rest and Exercise in the Diagnosis and Management of Infectious Hepatitis. By RICHARD B. CAPPS and (by invitation) M. HERBERT BARKER, New York, N. Y.

Heretofore the influence of rest and of exercise on the course of infectious hepatitis has not been recognized. We believe that this is the first systematic investigation of this problem. During an extensive experience in the army we have personally studied in over 600 cases the effect on both the clinical and laboratory picture of a 10-day graduated exercise tolerance test. In addition, observations have been made on comparable groups treated with different degrees of rest.

We have found that when active hepatitis is present, exercise will produce an increase in liver size, liver tenderness, symptoms and in the laboratory evidence of liver dysfunction. These findings persist for days or weeks and constitute a true exacerbation of the disease even with recurrent jaundice.

These observations demonstrate (1) that early and adequate bed rest is a most important therapeutic measure; (2) that recovery from this disease is not coincident with the disappearance of jaundice, and that the effect of exercise is a valuable criterion of recovery; (3) that the degree of liver enlargement and tenderness and the severity of symptoms at a particular time is related to the rest or exercise status of the patients; and (4) that an exercise tolerance test is a valuable diagnostic procedure but must be employed with discretion.

Action Spectrum of Ultraviolet Keratitis. By DAVID G. COGAN and (by invitation) V. EVERETT KINSEY, Boston, Mass.

The present study consists of a quantitative determination of the ultraviolet bands capable of producing an abiotic reaction in the cornea and a comparison of the radiations which produce a keratitis with those which produce erythema. For the cornea the action spectrum was found to have a sharp peak at 288 mu. which is not materially different from the corrected peak for skin. But whereas there is no substantial difference in the absorption spectrum and action spectrum of skin, there is a difference in the absorption spectrum of the cornea and the action spectrum of keratitis. It is concluded that the absorption spectrum of the cornea is largely determined

by the nucleoprotein (peak at 265 mu.) while the action spectrum is determined by a photolabile substance with absorption properties shifted toward the longer wave lengths (peak at 288 mu.). The action spectrum of keratitis is compatible with the assumptions that the photolabile substance is either one of the cytoplasmic proteins (albumen or globulin) or a specific portion (amino acids containing phenyl rings) of the nucleoprotein.

Since ultraviolet keratitis is a common occupational hazard (e.g. among welders), the transmission characteristics of various types of common glass were measured for that portion of the spectrum which is responsible for keratitis. By comparison of these characteristics with the action spectrum for keratitis, it is possible to determine the amount of protection provided by any one type of glass.

The Absence of Rapid Deterioration in Moderately Active Young Men on a Diet Restricted in Vitamins of the B Complex and Animal Protein. By ROBERT C. COGSWELL, GEORGE BERRYMAN, CHARLES R. HENDERSON, CHARLES W. DENKO, JANE SPINELLA, THEODORE E. FRIEDMANN, and ANDREW C. IVY (introduced by John B. Youmans), Nashville, Tenn.

Following a twelve-week period on an "adequate" diet, seven normal young men were fed for five weeks on a diet containing 0.16 mgm., 0.11 mgm. and 2.1 mgm. of thiamine, riboflavin and niacin, respectively, per 1000 calories of food consumed. The intake of the various lesser known B complex vitamins (para-amino-benzoic acid excepted) ranged from 28 to 66 per cent of that in the "normal" diet. The daily intake of protein was 48 grams, of which approximately 34 per cent was nonanimal. Corn (maize) comprised 27 per cent of the total calories of the diet.

The effect of this dietary was reflected in one week in the level of urinary excretion of the various vitamins, all of which were greatly reduced. No decrease occurred at any time in the content of the vitamins in the feces, however. No effect on group averages of physical or psychomatic response was observed; but, examination in retrospect of certain of the individual measurements of physical efficiency indicate the possibility that in two individuals slight beginning changes may have occurred in this function though not in the tests measuring psychomotor function. These individual changes, while not statistically significant for the five-week period do, however, comprise a part of the statistically significant change observed at the end of fifteen weeks of the experimental diet.

Low Oxygen Consumption and Low Ventilatory Efficiency During Exhausting Work in Patients with Neurocirculatory Asthenia, Effort Syndrome, Anxiety Neurosis. By MANDEL E. COHEN (by invitation), ROBERT E. JOHNSON (by invitation), FRANK CONSOLAZIO (by invitation), and PAUL D. WHITE.

Oxygen consumption was measured minute by minute while subjects ran to exhaustion on a motor driven treadmill at 7 m.p.h. at a grade of 8.9 per cent. Comparison was made among 20 patients with neurocirculatory asthenia (N.C.A.), average age 28 years; 20 healthy men, age 29; and 129 healthy young men, age 20 (Heath's subjects). Maximum oxygen consumption per kgm. body weight and minute averaged 36.8 ml., 47.0 ml., and 51.8 ml. respectively. To rule out the possibility that this difference occurs because N.C.A. patients do not run as long as healthy subjects, comparison of oxygen consumption in the 3 groups during the first, second and third minute of running showed a progressively greater difference; average values in ml. per kgm. body weight and minute were: N.C.A., 33.8, 40.0, 37.7; healthy 29 year old men, 35.0, 43.9, 46.5; healthy 20 year old men 38.4, 48.2, 50.5. In general, men who ran the longest had the highest rate of consumption of oxygen.

In a given individual the concentration of lactate in blood drawn 5 minutes after the run described above was directly proportional to the time of running, but the absolute value per second of running varies from individual to individual. For 68 N.C.A. patients, the average value was 1.31 mgm. of blood lactate per 100 ml. blood and second of running; and for 46 healthy men of comparable age, 0.60.

Ventilatory efficiency (V.E. = (oxygen consumed)/pulmonary ventilation), was lower at all times in N.C.A. Average values in ventilatory efficiency were: N.C.A., 4.29, 3.88, 3.46; healthy 29 year old men, 5.42, 4.59, 3.92.

It is concluded that for the same amount and duration of hard work, N.C.A. patients consume less oxygen, have a lower ventilatory efficiency and show a higher blood lactate than do healthy control subjects of comparable age. The data are all consistent with the idea that aerobic metabolism in hard muscular work is abnormal in N.C.A. and suggests high oxygen debt.

These data do not answer the question whether this is a phenomenon of poor health in general, poor running ability or lack of physical training.

Galactose Removal Constant. An Expression of Galactose Disappearance from the Blood Stream. By HENRY COLCHER (by invitation), ARTHUR J. PATEK, JR., and (by invitation) FORREST E. KENDALL, New York, N. Y.

The rate of disappearance of galactose from the blood stream after intravenous injection of 0.5 gram per kgm. body weight was studied in normal adults, patients with Laennec's cirrhosis of the liver, chronic passive congestion of the liver, acute hepatitis, and other affections of the liver.

A total of 89 determinations were made in 64 patients. In each patient the rate of disappearance of galactose was proportional to its concentration in the blood. The determination of blood galactose was based upon Benedict's method for blood sugar, after removal of glucose by fermentation according to Raymond and Bianco's method. It was shown that galactose determinations made on samples of blood drawn 15 and 45 minutes after intravenous injection enabled one to calculate the "Galactose Removal Constant." This constant expressed the per cent by which the concentrations fell each minute. It is calcu-

lated by the following equation:

$$G.R.C. = \frac{2.3 \ (\log C_1 - \log C_2)}{t_1 - t_2}.$$

Where C_1 and C_2 represent the concentrations at the times t_1 and t_2.

The Galactose Removal Constant varied between 4.2 and 9.5 in the control group of 10 adults, whereas it was below 4 in 43 patients with impaired liver functions. There was a good correlation between the changes in the Galactose Removal Constant and tests pertaining to other liver functions.

Metabolism of Thiourea: Additive Effects of Iodine and Thiourea in the Treatment of Hyperthyroidism. By THADDEUS S. DANOWSKI and EVELYN B. MAN (by invitation) and ALEXANDER W. WINKLER, New Haven, Conn.

The metabolism of thiourea has been studied in dogs and in humans. Thiourea is destroyed only in the kidney. It appears to exert its depressant effect on the thyroid gland without being destroyed itself in any measurable quantity. This destruction in the kidney is dependent, in certain patients, upon the level of metabolism; untreated myxedematous patients do not destroy the drug. There is no evidence, however, that in patients who are already capable of destroying thiourea this ability is enhanced in states with increased metabolism such as hyperthyroidism.

Serum precipitable iodine and basal metabolic rate were followed in 54 patients with hyperthyroidism under various forms of treatment. Iodine administered simultaneously with thiourea (0.2 gram daily) produced a more rapid and marked remission than did thiourea alone. Prolonged preliminary medication with iodine did not delay the response to thiourea. Omission of iodine medication in 6 patients maintained in remission on combined therapy for several weeks resulted in an exacerbation of hyperthyroidism in 4 of them. Thiourea and iodine medication supplement rather than interfere with one another in the treatment of hyperthyroidism, and should in general be given together.

Contrasts in the Effect of Blood Transfusions and of Liver Extract upon the Peripheral Blood and Bone Marrow in Pernicious Anemia. By CHARLES S. DAVIDSON, Boston, Mass.

Five patients with pernicious anemia in relapse were repeatedly transfused with red blood cell concentrates so that within a few days high red cell values were attained. Subsequently, liver extract was given intramuscularly. Transfusion was without detectable effect on reticulocytes, white cells, platelets, or significantly on the patients' clinical condition. In two patients whose red cells had reached 5 million per cu. mm., only slight reticulocyte increases occurred after the liver extract but white cell and platelet counts rose promptly to normal. In two patients whose red cell counts had reached about 3 million per cu. mm., moderate reticulocytosis occurred after liver extract and white cells and platelets returned to normal.

The fifth patient was transfused to 5 million red cells but further therapy was withheld for 54 days until his red cell count had fallen to 3 million per cu. mm. Liver extract administration was followed by typical responses of the reticulocytes, white cells and platelets.

In the three patients with red cell counts of 5 million per cu. mm. following transfusions, the bone marrow became more mature in erythropoietic elements, but the cells of the granulopoietic series appeared to be unchanged. However, following liver extract, administered to two of these patients, more mature granulopoiesis appeared. Thus, the megaloblastic bone marrow in pernicious anemia was significantly modified after artificial increase in its oxygen tension, while white cells and platelets responded only after liver extract.

Studies on Bacteria Developing Resistance to Penicillin Fractions X and G in Vitro and in Patients under Treatment for Bacterial Endocarditis. By HARRY F. DOWLING and (by invitation) HAROLD L. HIRSH and C. BARBARA O'NEIL, Washington, D. C.

Sixteen strains of bacteria were made resistant to penicillin X and penicillin G by serial passage in media containing increasingly larger amounts of these fractions. At the termination of the experiments, those strains whose resistance had been raised to penicillin X were relatively more sensitive to penicillin G and those strains whose resistance had been raised to penicillin G were relatively more sensitive to penicillin X. In every instance when the resistance to one fraction was raised, the resistance to the other fraction followed along, to the same or nearly the same extent.

The sensitivity of the etiologic organisms to penicillins X and G were studied in a patient with *Streptococcus viridans* endocarditis and in a patient with *Staphylococcus aureus* endocarditis. In each instance, while the patient was receiving penicillin, the organism responsible for the infection developed resistance to both penicillin fractions simultaneously.

It is concluded that organisms which cause human infections usually do not show great differences in their relative sensitivity to penicillin G and penicillin X. In most instances, when resistance to one of these fractions increases, resistance to the other fraction will also increase.

The Therapeutic Efficacy of Penicillin in Experimental Syphilis as a Function of the Method of Administration. By HARRY EAGLE and (by invitation) HAROLD J. MAGNUSON and RALPH FLEISCHMAN, Baltimore, Md.

The therapeutic efficacy of penicillin in experimental rabbit syphilis has been found to be profoundly modified by the interval between injections and their total number. Thus, the total curative dose was reduced from 60,000 units per kilogram to 360 units per kilogram by increasing the number of injections from 8 to 50, and a qualitatively similar decrease was effected by increasing the interval between injections from 1 to 4 hours. Further prolongation had no effect, up to the maximum time interval studied (1 to 4 days).

These results reflect the fact that the total time for which spirochetes are exposed to effectively spirocheticidal concentrations is of greater importance than the absolute tissue levels of penicillin. A quantitative formulation of these relationships has been derived which corresponds closely to the observed therapeutic efficacy of penicillin in 11 different treatment schedules.

If the results in experimental animals are applicable to man, there is reason to believe that penicillin may be given effectively on an ambulatory basis, as infrequently as once or twice daily, and perhaps even at irregular intervals. The only essential requirement is that the patient receive the requisite total number of injections within a reasonable period of time, provided they are not given so frequently as to produce cumulative effects on the blood penicillin level.

The Osmotic Fragility of the Red Cells of the Peripheral and Splenic Blood in Patients with Congenital Hemolytic Jaundice Transfused with Normal Red Cells. By CHARLES P. EMERSON, JR., and SHU CHU SHEN (by invitation) and WILLIAM B. CASTLE, Boston, Mass.

The red cells of patients with congenital hemolytic jaundice, before and after splenectomy, were found to be abnormally susceptible to destruction by hypotonic solutions and by trauma. Moreover, during *in vitro* incubation (erythrostasis), the osmotic and mechanical fragility of these cells increased at an abnormally rapid rate. Blood from the splenic vein, and particularly from the splenic pulp, exhibited a more pronounced increase in osmotic and mechanical fragility, and a higher proportion of such fragile cells, than did the peripheral blood.

Combined Ashby counts and osmotic fragility studies in patients transfused with normal blood several days before splenectomy indicated that the donor cells survived as in normal recipients. In the splenic pulp, however, the percentage of donor cells was significantly lower than in the peripheral blood, and the osmotic fragility of the donor cells, in contrast to the patient's cells, was essentially unaltered. Thus, the patient's red cells appear to be selectively retained and their fragility selectively increased in the spleen. It is concluded that the spleen contributes to excessive blood destruction in congenital hemolytic jaundice through the mechanism of erythrostasis, which results in abnormally rapid deterioration of the inherently defective red cells of the patient.

Hyperventilation: Mechanism of Symptoms. By GEORGE L. ENGEL, EUGENE B. FERRIS, and (by invitation) MYRTLE LOGAN, Cincinnati, Ohio.

Hyperventilation is an important neurotic symptom, occurring both as a physiological concomitant of the emotions of fear or anger, and as an hysterical manifestation. It is also seen during diffuse encephalopathies and during anoxia (altitude).

Study of patients and normal volunteers has revealed that there are two components to the symptomatology. Reduction in consciousness, with its associated symptoms of faintness, giddiness, fullness in head, etc., is correlated with change in frequency of the EEG, and this may be modified strikingly by changes in blood sugar, oxygen tension of inspired air, posture, and certain cerebral vasodilators. Tetany, on the other hand, is completely unrelated to changes in the electrical activity of the cortex and is apparently peripheral in origin. Of the two groups of symptoms, reduction in consciousness is much more common and more serious, both experimentally and clinically.

Studies of arterial and internal jugular venous blood have not been helpful in elucidating the mechanism of the changes in consciousness.

Although reduction in consciousness may be marked, actual syncope, with falling, is rare. Four mechanisms of syncope have been observed:

1. Vasodepressor syncope, concurrent or delayed;
2. Accentuation of already present orthostatic hypotension;
3. Hysterical syncope or convulsions;
4. Central type.

Differentiation of these types of fainting will be discussed.

Breathholding. Interchange of Gases between the Blood and Lungs during Voluntary Breathholding and the Factors which Influence the Maximum Breathholding Time. By EUGENE B. FERRIS, GEORGE L. ENGEL, CHARLES D. STEVENS, MYRTLE LOGAN, and JOSEPH P. WEBB, Cincinnati, Ohio.

It has been observed that during breathholding the buoyancy of subjects when submerged completely under water decreases at a constant rate which is only slightly less than the rate of total oxygen consumption of the body. The changes in buoyancy are measured by weighing the subjects as they lie under the water, suspended on a weighing pan. The loss of buoyancy or gain in underwater weight is shown to be due to loss of gas (chiefly oxygen) from the lungs.

By underwater weighing, serial study of the arterial blood gases and measurement of the effect of breathholding on the maximal expiratory lung volume, it has been determined that during breathholding the rate of diffusion of oxygen from the lungs into the blood is much faster than the rate of diffusion of carbon dioxide from the blood into the lungs under normal conditions. The higher the tension of inhaled oxygen in the lungs before breathholding, the more rapid is the rate of diffusion of oxygen through the lungs into the blood during breathholding. During breathholding, most of the carbon dioxide produced by the body remains in solution in the blood and tissue fluids and very little diffuses out into the lungs as a gas.

With respect to the duration of maximum time of voluntary breathholding, it has been determined that the stimulus to breathe is dependent upon arterial oxygen content, carbon dioxide tension and to some extent, on sugar content. At oxygen tensions of 75 to 150 mm. Hg in the inhaled air, the length of time of breathholding is directly proportional to the change in oxygen tension. At

higher oxygen tensions of inhaled air, and corresponding tensions of arterial blood there is still a relation, but the effect becomes less and less as the oxygen tension is raised. Serial studies of arterial blood gases and pH during breathholding with varying oxygen-nitrogen mixtures indicate that the decreasing effectiveness of higher oxygen tensions (above 20 per cent oxygen) in increasing the breathholding time is due to the fact that the capacity of the blood to carry oxygen to the respiratory centers is less influenced by raising oxygen tension once the hemoglobin is saturated.

With respect to the stimulus to breathe after maximum voluntary breathholding it is shown that arterial oxygen content and carbon dioxide are inversely and continuously interrelated as factors which influence respiratory activity over such a wide range of oxygen tension in inspired air as 75 to 750 mm. Hg.

These studies throw light on the interrelation of oxygen, carbon dioxide, and other metabolites as respiratory stimulants in man and offer a new approach to the study of pulmonary gas diffusion and respiratory activity in health and disease.

A Study of Congenital Methemoglobinemia. By CLEMENT A. FINCH, HOWARD A. EDER, and RALPH W. MCKEE (introduced by George W. Thorn), Boston, Mass.

A case of congenital intracellular methemoglobinemia was investigated. The patient, a 24-year old boy cyanotic since birth, when seen had a level of 40 per cent methemoglobin (Fe^{+++}).

With his hemoglobin initially converted into a bivalent state (Fe^{++}), the patient formed Fe^{+++} at a rate of 3 per cent a day. When his washed cells were transfused into a recipient, the rate of Fe^{+++} formation was precisely the same. This and *in vitro* studies indicated that the defect was localized in the red cell.

Methemoglobin was then produced by sodium nitrite. Whereas in the normal person Fe^{+++} is rapidly reconverted to Fe^{++}, in this patient there was a complete failure of this reconversion.

The ability of the erythrocyte to reconvert Fe^{+++} to Fe^{++} has been shown to be related to the glucose metabolism of the red cell. No apparent defect of respiratory enzymes, phosphorylation or glycolysis was found in this patient.

Methylene blue, an artificial intermediary between glucose metabolism and Fe^{+++} reconversion, was effective for three years in controlling this patient.

It was concluded that the patient has a biochemical lesion of the red cell, that the fault is one of reconversion of Fe^{+++} to Fe^{++}, and that his red cells lack a methylene-blue-like substance which normally links methemoglobin reconversion to glucose breakdown.

The Effect of Stress Situations on Lymphocytes in Psychoneurotic Patients. By JACOB E. FINESINGER and (by invitation) GREGORY PINCUS, MARY A. B. BRAZIER, and ARTHUR L. WATKINS, Boston, Mass.

The effect of stress situations on the lymphocyte count was studied in a series of psychoneurotic patients. The diurnal rhythm for each patient was established by determining the lymphocyte count at frequent intervals throughout a period of three successive days. A gradual increase in the absolute number of lymphocytes during the afternoon hours was found as has been previously observed in normal control subjects. The patients showed a decrease in the number of lymphocytes when they were interviewed and a disturbing topic was discussed for a period of one hour. This decrease was not observed when a casual topic was discussed with the same patients during an interview at the same time of day for a similar period. Other stress situations such as the exposure to cold for a period of one hour also decreased the lymphocyte count. Uric acid studies were done to determine whether the decrease in lymphocytes was due to a breakdown of lymphocytic tissue. It is concluded that the stress situation resulted in a relative lymphopenia, most probably caused by an increased secretion of adrenal cortical hormone.

Phosphorus Metabolism in Diabetic Coma. By MAURICE FRANKS, ROBERT F. BERRIS, and NATHAN O. KAPLAN (by invitation) and GORDON B. MYERS, Detroit, Mich.

On twenty-eight patients in diabetic coma, determination of blood inorganic phosphorous, glucose, chloride, CO_2 combining power, hematocrit and specific gravity were made at approximately four hour intervals for twenty-four hours. Quantitative urinalyses were made in parallel periods for phosphorus, chloride, glucose, and nitrogen. Blood phosphorus was invariably elevated on admission, and fell precipitously to subnormal levels in all but one case. Urinary phosphorus was subnormal after the first four hours.

Ten patients received either 1319 or 2638 mgm. of phosphorus intravenously as buffered sodium phosphate, injection beginning at 4.8 hours and completed within 2.4 hours. After a transient rise, plasma phosphorus fell to normal or subnormal levels despite the retention of most of the injected phosphorus. Distinct clinical improvement as judged by blood pressure, mental state, and CO_2 combining power occurred during phosphate administration in nine cases. Two died later of cardiac failure, one of pulmonary embolism, giving a fatality rate of 30 per cent compared with a predicted rate of 40.5 per cent, according to criteria of Collen. These cases were compared with seven treated according to method of Joslin with an expected fatality rate of 22.1 per cent and an actual rate of 14.3 per cent and with eleven cases treated with liberal glucose intake, having a predicted fatality rate of 34.8 per cent and actual rate of 27.3 per cent.

The Auriculo-Temporal Syndrome. By A. S. FREEDBERG, R. S. SHAW, and M. J. MCMANUS (introduced by M. D. Altschule), Boston, Mass.

In contrast to the extensive knowledge regarding denervation of sympathetic adrenergic nerves, little information is available concerning the effects of denervation of parasympathetic cholinergic fibres in man. The auriculo-

temporal syndrome affords an opportunity to study the latter. This syndrome is characterized by gustatory sweating on eating, and flushing over the cutaneous distribution of the auriculo-temporal nerve. Two patients have been studied who exhibited this syndrome many years following parotitis with incision and drainage.

Facial sweating and flushing were observed ten seconds or more after chewing food or tasting vinegar, but not after chewing paraffin or after psychic stimulation. During food stimulation no saliva was obtained in one patient from the involved parotid gland, while in the other patient, no differences in the salivary secretion on the two sides were observed. Previous cooling of the involved skin surface diminished or slowed, but did not abolish, the sweating.

In one patient, skin temperature measurements made during cooling and heating of the body showed a decreased response of the involved ear to warming. In the other patient after maximal response to heat occurred, chewing an apple resulted in profuse sweating over the involved cheek, and a sharp further increase in temperature of the involved ear. In both patients, heat sweating was diminished on the involved side.

The involved side showed sweating to significantly higher dilutions of acetyl-choline bromide introduced intradermally by means of a needle or by means of iontophoresis in each instance.

Procainization of the auriculo-temporal nerve on the involved side resulted in anesthesia and abolished the sweating reaction. In both patients after procainization of the superior cervical ganglion on the involved side, sweating occurred after eating.

The intravenous administration of 110 ml. of acetylcholine bromide (80 μg. per ml.) resulted in slight sweating on the involved side in one patient.

The conclusions drawn from this study are:

(1) The auriculo-temporal syndrome is a manifestation of a reflex in which the efferent arc is through the auriculo-temporal nerve or through cranial autonomic fibres adjacent to it, and not through the cervical sympathetic nerves.

(2) There is a deficiency in vasomotor, thermo-regulatory, and sensory innervation over the involved area.

(3) A local hypersensitivity of the sweat glands to acetyl-choline bromide is present.

(4) The mechanism of this syndrome is probably related to denervation hypersensitivity of the sweat glands in the involved area analogous to the hypersensitivity to adrenalin which occurs after post-ganglionic division of sympathetic fibres.

Quantitative Microdetermination of Estrogens by Ultraviolet Absorption Spectrophotometry. By HARRY B. FRIEDGOOD and (by invitation) JOSEPHINE B. GARST, Los Angeles, Calif.

A quantitative spectrophotometric method for assay of estrogens has been developed because of the gross inaccuracy of bioassay and colorimetric techniques. Ultraviolet absorption curves of chemically pure crystalline estrone, estriol and estradiol were determined at intervals of 2 millimicrons between 226 and 300 millimicrons with the Beckman Quartz Spectrophotometer. Concentrations of 0.125, 0.100, 0.075, 0.050, 0.025 and 0.0125 mgm. per ml. were used to determine the concentration-extinction relationship and individual calibration curves. Mixtures of crystalline estrogens were hydrolyzed and extracted by various techniques and assayed spectrophotometrically.

The estrone and estriol absorption curves exhibited minimum density or maximum transmission at 248 millimicrons and maximum density between 280–282 millimicrons with secondary peaks at 288 millimicrons. Another maximum density occurred below 230 millimicrons. Estradiol showed the same peaks except at the lowest extinction which occurred at 252 millimicrons instead of 248 millimicrons. The peak at 280 millimicrons was selected for the construction of calibration curves for each estrogen. The relation between density and concentration followed Beer's law between $E = 0.10$ and $E = 1.00$ at 280 millimicrons.

The identification and quantitation of estrogens through ultraviolet spectrophotometry and chemical separation has made it possible for the first time to analyze and criticize accurately the various techniques currently used for the extraction and separation of urinary estrogens.

The Anti-Anemic Effect of Synthetic Lactobacillus Casei Factor (Folic Acid) in Man. By GRACE A. GOLDSMITH, New Orleans, La.

Synthetic *L. casei* factor was administered to 2 patients with pernicious anemia, 4 patients with nutritional macrocytic anemia, and 1 patient with normacytic anemia with hypoplastic bone marrow. Marked clinical and hematological improvement occurred when 5 to 120 mgm. *L. casei* factor was given daily, either orally or parenterally. Reticulocytes increased 5 to 10 days after therapy was instituted, followed by a gradual rise in erythrocytes and hemoglobin to normal levels in 2 instances, and slightly below normal in 5. The bone marrow, whether initially hypo- or hypercellular, returned to normal in 4 patients in whom studies have been completed. When the initial leukocyte count was low, it increased during therapy.

Four patients have been maintained in good condition for 6 months with the oral administration of 15 to 30 mgm. of *L. casei* factor daily. One person with nutritional macrocytic anemia has been studied for 2 years. The therapeutic effect of liver extract, yeast, several B vitamins and *L. casei* factor will be compared.

Two patients with sprue and 1 with celiac syndrome were benefited by treatment with *L. casei* factor; diarrhea ceased, fat in the stools diminished and lingual papillae regenerated. The erythrocyte count increased in 1 patient, but remained at the initial level of 3.5 to 4 million in the others. Two patients with aplastic anemia and 1 with macrocytic anemia of unknown origin failed to improve when *L. casei* factor was administered.

L. casei factor has marked anti-anemic activity which resembles but is not identical with that of liver extract.

Alteration of Intermediate Metabolism in Hypoleydigism and in Hypopituitarism in Man. By JAMES A. GREENE, Houston, Texas.

The non-protein respiratory quotient was obtained by studying four patients with primary hypoleydigism, four patients with hypopituitarism and secondary hypogonadism, one patient with only hypopituitarism and a normal prepubital boy in an open circuit metabolism chamber for 24 hours after constant diets.

The non-protein respiratory quotient was reduced in all untreated patients, it returned to normal in two primary hypoleydigism cases following testosterone therapy, it was not altered by testosterone therapy in those secondary to hypopituitarism, but was increased to a certain extent following a pituitary extract treatment. It was depressed in a case of hypopituitarism without gonadal abnormality and was normal in a normal prepubital boy.

The decrease of the non-protein respiratory quotients when interpreted in the usual fashion indicate a diminished oxidation of carbohydrate in the metabolic mixture in cases of hypopituitarism or hypoleydigism and suggest that the pituitary gland is responsible for this abnormality. The alteration is an abnormality and not a normal prepubital condition.

The Mechanism of the Fall in Arterial Pressure Produced by High Spinal Anesthesia in Patients with Essential Hypertension. By RAYMOND GREGORY (introduced by George M. Decherd, Jr.), Galveston, Texas.

The problem has been investigated by repeated simultaneous recordings of arterial and venous pressures before and 'during high spinal anesthesia in 5 patients with normal blood pressure and in 12 studies on 10 patients with a clinical diagnosis of essential hypertension. The cardiac output studies with the acetylene method have been made before and during high spinal anesthesia in patients with normal blood pressures and in patients with clinical diagnosis of essential hypertension. The data show no uniformity in the changes in arterial and venous pressures. The venous pressure may fall without any fall in the arterial pressure. The arterial pressure may fall greatly for a number of minutes before the venous pressure falls. The venous pressure may rise to control levels while the arterial pressure remains at the lowest level of fall caused by the spinal anesthesia. It is concluded that high spinal anesthesia in patients with essential hypertension produces no uniform change in the relationship between arterial and venous pressures; that the changes in the arterial and venous pressures are not causally related; and that the fall in arterial pressure probably is not due to a diminished cardiac output produced by increased venous return.

The Incidence of Penicillin Resistant Staphylococci in War Wounds in Relation to Penicillin Therapy. By J. D. HAMILTON, T. E. ROY and L. GREENBERG (introduced by Donald McEachern), Montreal, Canada.

A series of 503 war wounds, 4 to 20 days of age, were examined bacteriologically at Base Hospitals in Italy. These comprised two groups: One had previously received parenteral penicillin therapy as a prophylactic measure; the second had received no penicillin.

The total dose of penicillin averaged 450,000 units per case given in 3-hourly intramuscular injections of 15,000 units. Cultures were taken only after arrival at Base Hospital, and in the treated group this was from 2 to 14 days after the end of penicillin therapy.

The incidence of pathogenic Staphylococci was not greatly reduced in the penicillin treated group (33 per cent) compared to the control group (37 per cent). There was a marked difference in the proportion of penicillin resistant strains. In the penicillin treated group 49 per cent of strains were resistant and 17 per cent in the control group. Older wounds showed the same differences as the younger ones.

The elimination of sensitive strains from some of the treated wounds, hospital cross infection with resistant strains, and penicillin resistance induced *in vivo* are discussed as factors concerned, but no single factor adequately explains the findings. The difficulty of interpretation is probably related to the interaction of many factors.

The Effect of Roentgen Therapy on Experimental Ocular Vaccinia in Non-Immune and Partially Immune Rabbits. By GEORGE T. HARRELL and (by invitation) HAL W. PITTMAN, LAWRENCE B. HOLT, CHARLES H. REID, J. M. LITTLE, JAMES W. MANKIN, and LESLIE MORRIS, Winston-Salem, N. C.

An accidental human ocular infection with vaccinia secondary to inoculation of the arm was treated with roentgen therapy without the development of residual scarring. The response led to investigation as to whether immunity or roentgen rays were responsible for the improvement. Primary ocular vaccinia and that secondary to inoculation from a separate site have not always been differentiated; hence, the role of immunity cannot be established from the literature. Preliminary experiments suggested that roentgen therapy to primary corneal vaccinia in rabbits diminished the residual corneal opacities and hastened the regression of the acute lesions. Best results were obtained when irradiation was given after the lesion appeared and before it reached its height. In subsequent experiments groups of 15 rabbits were used: (*1*) primary infection, treated with x-ray (*2*) primary infection, untreated; (*3*) secondary infection after inoculation of the flank, treated with x-ray; (*4*) secondary infection, untreated; (*5*) primary infection with subsequent inoculation of the flank; (*6*) trauma of inoculation without infection, treated with x-ray—negative control. Opacities of the cornea were measured by a photoelectric colorimeter. Roentgen therapy did not markedly reduce residual corneal opacities resulting from primary or secondary inoculation. The partial immunity produced by preliminary inoculation of the flank three days before ocular inoculation did not reduce residual corneal opacities. Secondary inoculation of the flank three days after ocular inoculation did not reduce corneal opacities.

Experimental Serum Sickness: Relationship Between the Nature of the Antigen, the Serologic Response, and the Pathological Change in Rabbits Given Purified Bovine Plasma Proteins. By CLINTON VAN ZANDT HAWN and WILLIAM C. BATCHELOR (by invitation) and CHARLES A. JANEWAY, Boston, Mass.

Two different purified fractions of bovine plasma have been injected into rabbits and the fate of the injected protein followed by quantitative immunologic methods. Whereas crystallized bovine serum albumin (CBA) disappears rather slowly from the circulation, bovine serum gamma-globulin (BG) disappears within two weeks of injection. This slow rate of disappearance of CBA has also been observed in dogs and humans.

These differences are paralleled by differences in antigenicity. Both bovine serum and BG appear to be powerful antigens whereas CBA is much less antigenic. The pathological lesions found in rabbits after single large intravenous injections of these proteins differ in extent and distribution, CBA giving rise predominantly to generalized arteritis, and BG to lesions in liver and renal glomeruli. It is possible to correlate both the occurrence and the stage of the lesions with the serologic response—healing occurring when antigen disappears from and antibody appears in the blood.

These observations provide further experimental proof that anaphylactic reactions of the serum sickness type give rise to inflammatory lesions and that the character of the lesions is dependent on the chemical nature of the inciting antigen.

Circulatory Adjustments during Treatment with Large Doses of Sodium Salicylate or Acetylsalicylic Acid. By HANS H. HECHT and B. V. JAGER (introduced by Maxwell M. Wintrobe), Salt Lake City, Utah.

Thirty-five patients received a total of 41 courses of salicylate therapy. Each course was at least of 4 weeks' duration. The plasma salicylate levels were maintained at 250 μg. per ml. or higher. In 32 of these patients an average initial drop of 10 per cent in the volume of packed red cells (venous blood) was observed. The maximum reduction occurred during the first two weeks. Levels equal to or higher than those found prior to treatment were usually observed when salicylate therapy was discontinued. In the presence of anemia, however, an initial reduction in volume of packed red cells was frequently not obtained. In 4 patients with cardiac enlargement and in one without organic heart disease, the onset of pulmonary edema during treatment coincided with the reduction in the hematocrit values. Two of the patients in whom high plasma salicylate levels were obtained died with signs and symptoms of congestive failure which were not apparent when therapy was started. In 100 rabbits which died from salicylate intoxication, gross postmortem studies revealed acute congestion of the lungs, liver and spleen in all instances.

A detailed examination of the cardiovascular system before, during and after treatment with large doses of salicylates in 10 subjects who received 13 courses of therapy revealed a decided increase in the circulating plasma volume during therapy, as determined by chromatographic analysis of dye-plasma mixtures. The average increase in circulating plasma volume amounted to 22 per cent. A change in the opposite direction was noted when treatment was discontinued (average decrease 19 per cent). The average increase in the total circulating blood volume, computed from the plasma volume and hematocrit readings was less striking, though apparent in all cases. This was expected as the circulating red cell mass was not significantly altered in all instances. A great increase in circulating total blood volume was noted in cases with anemia where both the plasma volume and the red cell mass increased considerably under treatment. That the alterations observed did not represent changes in distribution of plasma and red cells in various parts of the venous system was suggested by the observation that simultaneous arterial and venous hematocrit readings were identical.

These cardiovascular changes were associated with a slight decrease in the circulating total proteins and a somewhat greater change in the total circulating albumin. There were no consistent changes in weight, urine volume, venous pressure or vital capacity or in the size of the thiocyanate space. No alterations of plasma and urine sodium concentrations were noted before, during or after treatment in 5 patients maintained on a controlled sodium intake. No difference in response was noted when sodium salicylates were replaced by acetylsalicylic acid.

The changes observed point to possible deleterious effects resulting from treatment with massive doses of salicylates. The increase in circulating fluids apparently is not accompanied by a definite increase in the extracellular fluid space and is not associated with sodium retention. It differs, therefore, from the alteration in fluid balance observed in congestive heart failure.

Elevation of Serum Precipitable Iodine During Pregnancy. By MARTIN HEINEMANN, CARL E. JOHNSON and EVELYN B. MAN (introduced by John P. Peters), New Haven, Conn.

Circulating thyroid hormone is measured more accurately by determinations of serum precipitable iodine than of basal metabolism. Since the latter increases during pregnancy, serum precipitable iodines were investigated in pregnant women. Their histories and physical findings excluded thyroid abnormality. Compared with a mean of 5.6 ± 1.3 μg. per cent in approximately 75 normal women, 35 determinations in 16 normal pregnant women ranged from 6.2 to 11.2 with an average of 8.4 μg. per cent, concentrations otherwise indicative of hyperthyroidism. This increase was noted as early as the third week of pregnancy and subsided within two weeks after delivery. It is not, therefore, correlated with the rise of basal metabolism which is a phenomenon of late pregnancy.

The implications of increased serum iodines during pregnancy are not clear. Thirty drops daily of Lugol's solution for one month did not diminish elevated serum

iodines in 2 cases. Iodine concentrations were identical in umbilical and maternal venous serum at birth in 2 instances. Of two pregnant females with serum iodines less than those of the 16 normals, one miscarried for unknown reasons during the third month. The second threatened to miscarry in the fifth month but continued pregnancy after intravenous administration of thyroxin.

Elevated serum iodine concentrations seem to be physiologic during pregnancy.

Folic Acid in Pernicious Anemia. Studies on Effect, Mechanism of Action, and Excretion. By ROBERT W. HEINLE and (by invitation) ARNOLD D. WELCH and EVELYN M. NELSON, Cleveland, Ohio.

Although no failure in treating pernicious anemia in relapse with synthetic folic acid (2 to 100 mgm. daily) has been reported, only minimal reticulocyte responses occurred in one patient following each successive period of intramuscularly administered folic acid: 1, 6 and 12 mgm., respectively. With continued vitamin administration, xanthopterin given orally appeared to produce a fourth small response. Since 15-unit liver extract subsequently produced a theoretically maximal response, factors accessory to folic acid may be involved in certain cases.

A less than theoretically maximal, delayed response occurred in another patient with pernicious anemia in relapse, after daily intramuscular administration of synthetic folic acid (5 mgm.). Xanthopterin did not perceptibly augment the effect of the vitamin.

A third patient, extremely sensitive to liver extracts, relapsed while taking 16 'Extralin' capsules daily. Forty mgm. of synthetic folic acid daily by mouth caused remission.

Normal gastric juice, duodenal content, or both together, did not split *L. casei* factor from purified conjugated folic acid. Studies on therapeutic activity and human enzymic splitting of the conjugate are in progress.

The daily urinary excretion of *L. casei* factor in treated patients varied widely: oral—9 to 24 per cent; parenteral—15 to 75 per cent.

Radioactive Iodine Treatment of Graves' Disease—A Progress Report. By SAUL HERTZ, Boston, Mass., and (by invitation) ARTHUR ROBERTS, Cambridge, Mass.

Before the meeting of this society in May, 1942, a preliminary report by the authors presented data on their first trials of radioactive iodine in the therapy of patients with Graves' disease. Since that time, additional cases have been treated and a follow-up on the entire series of thirty-one (31) cases is presented in this report. The results are analyzed in the light of the entire experience at the Massachusetts General Hospital and elsewhere to date of January 15, 1946, with respect to the application of atomic energy for the internal Beta-irradiation of the hyperplastic thyroid of these subjects.

Clinical data are given which indicate strongly the therapeutic efficacy of this method in the treatment of Graves' disease by non-surgical means. The specific manner in which the therapy may be practically employed

in a relatively inexpensive, highly effective, and safe program is outlined. The prediction is made that this method may displace the surgical ones now in vogue for the treatment of this disease when atomic energy sources are made readily available for controlled medical usage. The advantages and disadvantages of the several techniques which have been employed to date in this therapeutic program are discussed.

Quantitative Studies in Man of the Removal of Bromsulphalein from the Blood. By F. J. INGELFINGER, Boston, Mass.

After bromsulphalein, 150 mgm. per sq. m. surface area, is injected intravenously, its disappearance rate from the blood of normal subjects is such that a constant percentage of the dye present in the blood is removed per minute (between 10 per cent and 15 per cent per minute). When similar doses are given to patients with extra- or intrahepatic biliary obstruction, or when larger or repeated doses are given to normal subjects, the disappearance rate progressively decreases, possibly as a result of saturation of the removal mechanisms. In cases of cirrhosis without jaundice, the disappearance rate remains fairly constant, but ranges between 1.5 per cent and 5 per cent per minute.

When a constant intravenous infusion of bromsulphalein is given immediately after an initial injection of 50 to 200 mgm., a constant blood level of the dye can be achieved if less than 3 mgm. per sq. m. is infused per minute. This figure is not appreciably affected by the size of the initial dose, although the height of the constant level achieved varies with the amount initially injected.

Others have suggested that bromsulphalein is removed from the blood in two stages: a rapid take-up in a storage space, and a slower excretion by the liver. These studies support this suggestion and indicate that the maximum excretory rate of the liver approximates 3 mgm. per sq. m. per minute.

Tropical Nutrition. Biochemical and Clinical Observations on Troops. By R. M. KARK and W. B. BEAN and (by invitation) H. F. AITON, C. R. HENDERSON, E. D. PEASE, R. E. JOHNSON, and L. M. RICHARDSON, Ottawa, Can., Ft. Knox, Ky., Chicago, Ill., and Boston, Mass.

During 1945 two separate teams who trained together and used almost identical methods made systematic observations on the nutrition, health and fitness of troops in the tropics. The U. S. team studied 600 men in the Pacific; and the Canadian, 1019 Indian soldiers fighting in Burma. Data on environment, work, catering and food intakes were collected. Medical examinations and fitness tests were administered. Samples of the urine and blood of each subject were analyzed in specially constructed airborne field laboratories for hemoglobin, protein, salt, vitamins and acetone. The results could be interpreted by comparison with data collected in a similar way under rigidly controlled field conditions in North America.

Effects of Stress with a Note on "Tropical Deterioration." With stress men deteriorate similarly in tropical, temperate or cold environments. When diet was ample

and tropical disease well controlled, even white troops were efficient and healthy after two years of arduous field service.

Interrelations among Dietary Intake, Biochemical Measurements, Physical Efficiency and Clinical Findings. Close correlations existed between calculated dietary intakes and biochemical measurements. Few statistically significant correlations were found among biochemical values, fitness scores and clinical findings.

Nutritional Requirements for the Tropics. No support is given to the proponents of a large intake of vitamins or a low intake of animal protein.

Acute Osteoporosis of Disuse: A Metabolic Study. By F. RAYMOND KEATING, JR., MARSCHELLE H. POWER, H. HERMAN YOUNG, and HADDOW M. KEITH (introduced by J. H. Means), Rochester, Minn.

A 15-year-old boy who had so-called vitamin D resistant rickets was immobilized by a plaster cast from the waist down after osteotomies on both femurs. Metabolic balance studies were conducted during immobilization and following resumption of activity. Diet and excreta were analyzed for calcium, phosphorus and nitrogen, and frequent analyses of the blood and periodic roentgenograms of the skeleton were made.

Marked hypercalcemia and hypercalcinuria, accompanied by marked reduction in the concentration of alkaline phosphatase in serum, occurred during immobilization. These data suggested that immobilization had seriously impaired osteoblastic activity and permitted rapid demineralization of the skeleton. Roentgenograms showed the demineralization to be pronounced and largely limited to the legs and lower part of the spinal column. The hypercalcemia probably occurred because calcium was released by demineralization at a rate exceeding the maximal capacity of the kidney to excrete it.

During a period of increasing physical activity, the level of calcium in serum decreased and that of alkaline phosphatase rose concurrently, but hypercalcinuria persisted until calcium in serum had returned to normal levels. Further orthopedic surgery permitted additional studies throughout a second prolonged period of immobilization. The intense changes previously noted did not recur. The significance of these observations is discussed.

Oxidation and Reduction Systems in Articular Cartilage. By FRIEDRICH KLEMPERER (introduced by William W. Beckman), Boston, Mass.

Although respiration of articular cartilage is negligible, it was shown by Bywaters that in the presence of methylene blue appreciable amounts of oxygen are consumed. Hills found that this reaction was accompanied by CO_2 production and, without convincing experimental evidence, postulated that in the presence of methylene blue, cartilage is able to oxidize lactate to pyruvate, which is then decarboxylated with the formation of acetic acid. In the investigation to be reported it was impossible to demonstrate that oxidations that form part of the glycolytic cycle account for the reduction of methylene blue by bovine cartilage. Concentrations of iodo-acetate which inhibit glycolysis completely do not decrease the rate of methylene blue reduction, thus disproving the possibility of a reaction of phosophorylated glyceric aldehyde and methylene blue. In the presence of iodo-acetate, no formation of lactic acid was observed. Nevertheless, under these conditions the dye-induced oxygen uptake did not decrease the small amount of preformed lactic acid, and the addition of lactic acid had no accelerating effect on the reaction. Thus it would appear that lactic acid is not the substrate of methylene blue reduction. Neither glucose nor glycogen was oxidized by methylene blue in the presence of cartilage, and ammonia was not formed. Coenzymes, which are necessary for the maintenance of glycolysis in frozen and ground cartilage, were not involved in the oxygen uptake catalyzed by methylene blue. The reaction was not inhibited by fluoride arsenate or selenite but was abolished by heating and by caprylic alcohol. The mechanism for reduction of methylene blue in cartilage remains unexplained.

The Pathogenesis of Renal Failure in Patients with Severe Thermal Burns. By S. M. LEVENSON, W. E. GOODPASTOR, C. C. LUND, and F. H. L. TAYLOR (by invitation), and H. J. TAGNON, Boston, Mass.

Lasting impairment of renal function is seen frequently in patients dying as a result of severe thermal burns, and often contributes to the fatal outcome. In an attempt to elucidate the pathogenesis of this serious complication, a detailed clinical and pathologic study was carried out in 47 patients with fatal thermal burns.

A high correlation was found between clinical evidence of kidney dysfunction and significant renal changes observed at post mortem. Lasting impairment of renal function, as evidenced by persistent decrease in clearance on nonprotein nitrogen products, azotemia and oliguria or anuria persisting beyond the period of shock, was associated with definite morphologic changes at post mortem examination. These changes consisted principally of pigmented casts, epithelial casts, tubular necrosis, and, in cases surviving 48 hours, the beginning of tubular regeneration. The pigment casts gave positive staining reactions for red blood cell pigments. Patients with normal kidney function showed no significant renal changes at autopsy.

Hypoproteinemia, alkalinization, and toxic effects of sulphonamides, mercurial preservatives in plasma, or blood transfusion reactions were ruled out as contributing to the renal damage. The important etiological factors were: extent of third degree burn (over 30 per cent of the body surface), deep and prolonged shock, and hemoglobinuria consequent to the hemoglobinemia which was constantly seen in patients with severe burns.

It appears that under the conditions of prolonged renal ischemia, secondary to severe shock, the products of hemolysis are "toxic" to the kidneys, leading to both clinical and pathologic evidence of renal damage.

A Small Visual Comparator for the Rapid Determination of Plasma Volume. By ALICE LOWELL, FOREST E. KENDALL and WALTER MEYER (introduced by David Seegal), New York, N. Y.

A small visual comparator ($3.75 \times 3.75 \times 1.75$ inches) has been developed for the rapid determination of plasma volume. Measurements are made on serum samples taken before and 10 minutes after the intravenous injection of a known amount of the blue dye, T–1824. Repeated measurements can be made in the same patient.

This instrument has been used in 108 plasma volume determinations covering a range between 1300 and 6000 ml. The validity of the results obtained was checked by measurements made with a Bausch and Lomb spectrophotometer. The values derived from the two methods agreed within the margin of error inherent in plasma volume determinations. The average difference was 0.065 per cent; the standard deviation, 3.15 per cent.

This inexpensive instrument, because of its simplicity and compact size, can be used at the bedside or in the hospital emergency ward. It was originally developed to meet a possible need in an appropriate Army medical unit.

The Functional Capacity of Thyroid Tumors as Judged by Radioactive Iodine Uptake. By JANET W. MCARTHUR (by invitation) and OLIVER COPE, Boston, Mass.

Radioactive iodine in tracer dosage was administered preoperatively to eighteen patients with discrete nodules of the thyroid. The radioactivity of digests of the tumor tissue was determined and compared with that of the adjacent uninvolved tissue.

Of the eighteen tumors, twelve were benign. They included four involutional nodules, four struma nodosa micro et macrofolliculare, two fetal adenomas, one papillary adenocystoma and one Hurthle cell adenoma. The iodine collected by the less differentiated tumors, such as the fetal adenomas and Hurthle cell adenoma, was one-fiftieth to one-hundredth of that collected by the uninvolved portion of the gland. Four of the differentiated tumors collected more iodine than the uninvolved tissue, one twenty times as much. Two other differentiated tumors absorbed all of the iodine, the uninvolved tissue none; the patients had mild hyperthyroidism.

The six malignant tumors included papillary adenocarcinoma, metastasizing struma nodosa and adenocarcinoma. These collected from none to one-fourth of the radioactive iodine taken up by adjacent uninvolved tissue. In three instances there was a detectable collection of iodine by the tumor metastases.

The Circulation at Rest during Convalescence. By GEORGE R. MENEELY, E. BUIST WELLS and ALBERT SEGALOFF (introduced by Hugh J. Morgan), Nashville, Tenn.

The following measurements were made upon a group of convalescent patients who had undergone infectious dis-
ease or sur... ...tion... ...ht, pulse ...ira-

diameter of the heart and cardiothoracic ...
consumption, basal metabolic rate, ventilation
utilization coefficient, hematocrit value, total bl<
plasma volume and cell volume, plasma tota
total circulating plasma protein and an electroc
All studies were done under the basal conditi<
of these observations indicated any characteri
mality of the circulation when the results wer<
with currently accepted normal standards. Ind
viations were frequent but were understandab
as manifestations of the disease from which
had suffered, for example, anemia following
malaria. One may therefore conclude that n<
tests are of any use as a routine procedure for
classification of convalescence.

Follow up studies revealed small but impor
tions from "normal" which could only be <
comparing the individual with himself as a cont
differences were approximately one-half liter
ume deficit and approximately 35 per cent venoi
deficit during convalescence. It was also obs
the patients gained an average of 3.87 kilogr

*The Treatment of Lymphoblastic Leukemia w...
Myelokentric Acid.* By F. R. MILLER and (by
tion) H. W. JONES and P. A. HERBUT, Philadelph

Crude myelokentric acid was extracted from the
or feces of patients with chronic myeloid leukem<

The material was emulsified with alkaline-wate
pH of 7.5. After sterilization it was given to sev<
tients with lymphoblastic leukemia. This was done
effort to stimulate myeloid proliferation.

Twelve partial remissions occurred in treatin<
seven patients. These consisted of diminution of th
of lymph nodes and spleens, improved appetites ar
crease in body weight, as well as peripheral blood ch<
A shift from a lymphoblastic to a lymphocytic bloo<
ture occurred and was followed by a moderate to m
leukopenia, and then by the appearance of myeloid
including reticulocytes and platelets in the peri<
blood.

Eventually all died and five necropsies were obtai
All organs exhibited less leukemic infiltration than
treated cases. The infiltrations were not uniform a<
lymphoblastic leukemia but exhibited considerable p
morphism, an increase in reticulum cells, reticulum
also a few cells resembling Sternberg cells. The b
marrows appeared almost hypoplastic and the li<
showed a "washing out" of lymphoid elements.

Conclusion: Partial remission occurred with this tr<
ment. The necropsy findings might be interpreted
either beginning healing or changes simulating the o<
of Hodgkin's disease.

*Comparative Studies of the Trea<
dosis with and witho... Glucose<
(by invi... ...<
liver function<*

physiological or anatomical defects, permitting diuresis of water.

the Mechanism of Nausea. The Relation of Function to Nausea in Man. By STEWART New York, N. Y.

Experiments carried out during military service in which, nausea was induced in healthy subjects while activity of the first portion of the duodenum being observed by (1) recordings from an inlying balloon, (2) fluoroscopic examination of an inlying fast-filled balloon and (3) fluoroscopic examination of um-filled duodenum.

Nauseating stimuli were (1) irrigation of the ex-ear with cold water, (2) chemical emetics and (3) case of one individual, a fear-producing situation. nausea and vomiting were induced at will in this soldier discussion of either the horrors of the battlefields terrors of the jungle was initiated. In all subprofound changes in the pattern of activity of the um occurred coincident with the experience of however aroused. There was a narrowing of the of the first portion of the duodenum and the pre-pattern of normal contractions was interrupted replaced by contractions of slower frequency and ger duration.

Coincident with the subsidence of the experience of nausea these changes disappeared and the normal phasic rhythm of duodenal contractions was resumed.

The changes described were never observed in the absence of nausea and conversely nausea was never experienced without them.

Surface Phagocytosis—Its Relation to the Mechanism of Recovery in Pneumococcal Pneumonia. By W. BARRY WOOD, JR., and (by invitation) MARY RUTH SMITH, St. Louis, Mo.

A systematic study of the effect of chemotherapy upon the pulmonary lesion of experimental pneumococcal pneumonia has revealed that pneumococci are destroyed in the lung by phagocytosis and that the phagocytic process takes place in the absence of antibody. Further study has demonstrated that the phagocytosis is due to a hitherto undescribed mechanism which is not related either to opsonins or capsular injury but is dependent upon access of the phagocytes to a surface of suitable physical properties. Not only did all tissue surfaces tested support phagocytosis of virulent pneumococci, but even the surfaces of such inert materials as filter paper, cloth and fiber glass rendered the phagocytes highly active.

Direct visualization of the surface phenomenon shows that the phagocytic cells first pin the pneumococci against the surface before engulfing them. Those pneumococci which remain floating freely in the fluid medium are never successfully attacked except in the presence of specific opsonins. The non-antibody phagocytic process appears to be directly related to the mechanism of recovery in pneumococcal pneumonia and offers an adequate explanation for important questions hitherto unanswered by the conventional techniques of immunology.

Use of Transfusions in the Study of Hemolytic Anemia. By LAWRENCE E. YOUNG and JOHN S. LAWRENCE, Rochester, N. Y.

Transfusion studies employing the technique of differential erythrocyte agglutination provide a valuable means for investigating *in vivo* the destructive processes operating in certain types of hemolytic anemia. Studies of 2 unusual cases of chronic non-spherocytic hemolytic anemia are cited as illustrations.

In Case 1 phlebotomy was performed twice, and was followed immediately by transfusion of normal red cells. Donated normal cells survived normally in the patient's circulation but the patient's erythrocytes, 73 per cent of which were reticulated, were rapidly destroyed after transfusion to a small child. It was demonstrated, however, that the reticulocytes were not destroyed until they had become mature and that the *in vivo* maturation time was approximately 6 days. The patient's plasma had no demonstrable hemolytic effect on the red cells of a normal adult recipient. It is concluded that in this case the abnormality was in the erythron.

In Case 2 all red cells received from 30 donors were rapidly destroyed, possibly because of the action of cold hemagglutinins and alpha-one agglutinins of high thermal amplitude. It is considered likely that in this patient, who responded well to splenectomy, the abnormality was in the reticulo-endothelial system.

A conjectural classification of "intrinsic" hemolytic anemias on the basis of results of transfusion experiments is suggested.

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TABLE II

Correlation of antifibrinolysin titers with antistreptolysin titers in well soldiers

| | Antifibrinolysin titer of initial sera | | | | | | | | | | | | |
	<50	50	100	150	200	300	400	500	600	800	1000	1400	Totals
<50	7	1	1										9
50	7	4	3		1								15
62	19	3	3	3	1								29
83	14	5	3	2				1					25
100	20	10	5	5	2								42
125	27	11	6	14	3	2							63
159	19	12	6	8	5	2	3	1					56
200	20	10	5	3	1	1	2				1		43
250	12	10	9	8	4	1	1	2					47
317	9	7	5	5	1	1	1		1	2	1	1	34
400	4	6	1	5	4								20
500	1	1	3	2	1	2	1		1				12
625		3		1						1			5
833	1	1											2
1000		1				1							2
Totals	160	85	50	56	23	10	8	4	2	3	2	1	404

(Left axis: Antistreptolysin titer of initial sera)

significant difference in the distribution of titers among the soldiers harboring group A as compared with other groups of β-hemolytic streptococci.

As shown in Table I, 11 per cent of sera in the subjects without evidence of recent streptococcal infection exhibited titers of antifibrinolysin greater than 150 units. The cause of the increased resistance in the sera of these subjects was not known, although the observation is in agreement with studies made on normal subjects using the method of Tillett and Garner for estimation of antifibrinolysin (8).

In order to obtain evidence concerning the specificity of elevated antifibrinolysin titers in normal subjects, a comparative study was made of the antistreptolysin and antifibrinolysin levels of the initial samples of sera collected from 404 healthy soldiers. These antibody titers are recorded in Table II. In general, the data showed that a high antifibrinolysin titer was associated with an elevated antistreptolysin titer. For example, there were 53 sera in which the antifibrinolysin titer was greater than 150 units, and in 43 of these the antistreptolysin titer was 159 or above. Since it is generally accepted that elevated antistreptolysin titers are indicative of past infection by the β-hemolytic streptococcus, the data presented in Table II suggest that elevated antifibrinolysin titers are due to previous streptococcal infection.

Previous investigations have shown (9) and the results of this study also demonstrated that a high antistreptolysin titer was not necessarily as-sociated with an elevation of the titer of antifibrinolysin antibodies. There were 221 subjects whose sera were found to have an antistreptolysin concentration of 159 units or greater, and only 43 or 19 per cent had an antifibrinolysin titer above 150 units. This would indicate, therefore, that many streptococcal infections, as evidenced by elevated antistreptolysin titers, are not followed by an elevation of antifibrinolysin antibodies.

Antifibrinolysin response to infection

That the plasma clots of subjects with hemolytic streptococcal infections exhibit resistance to fibrinolysis during convalescence is well established. However, the observed frequency with which antifibrinolytic properties may be demonstrated in the blood following infection caused by the streptococcus has varied considerably. This variation in the response of the host may be due to difference in (1) the methods used to measure antifibrinolysin, (2) the time in convalescence serum specimens are obtained for testing, (3) the criteria used to establish the diagnosis of a streptococcal infection, (4) the antigenic properties of the infecting strain of β-hemolytic streptococcus, (5) variations in the ability of the host to respond, and (6) the severity and nature of the infectious process.

In the present study an attempt was made to control the first three factors. A quantitative method (5) for the determination of antifibrinolysin antibodies was employed; antibody titers were determined on acute and convalescent sera at the

same time. Under these conditions an increase in either antistreptolysin or antifibrinolysin titer of two dilution increments was considered significant and beyond the error of the test (10, 5).

In order to identify streptococcal infections accurately, each subject admitted to the hospital was examined daily, multiple cultures of the throat were taken, and acute-phase and usually several convalescent-phase blood specimens were obtained.

It has been demonstrated (11) that antifibrinolytic properties may not develop until the second to fourth week of convalescence. With few exceptions, therefore, one or more serum specimens were taken during the third to sixth week following the initial bleeding.

Since it has been shown previously that bacteriological and clinical evidence alone is not necessarily sufficient to establish an etiological diagnosis (12), antistreptolysin as well as antifibrinolysin determinations were made on all sera collected. It was therefore possible to study the frequency and specificity of the antifibrinolysin response from the standpoint of the clinical diagnosis, the presence or absence of β-hemolytic streptococci obtained from cultures of the throat, and the antistreptolysin response.

Source of cases

The respiratory illnesses include (1) 116 consecutive hospitalized cases of sporadic exudative tonsillitis and pharyngitis studied in the spring of 1943 (12), (2) 100 cases of tonsillitis and pharyngitis resulting from a foodborne epidemic in November 1943 (13), and (3) 525 cases admitted to the respiratory wards in the winter and spring of 1944. Each subject was bled at the time of admission to the hospital, and one or more convalescent blood specimens were taken from 1 to 6 weeks later. On most hospitalized soldiers swab cultures of the throat were made on at least 3 successive days after admission. The clinical diagnosis was made from the history, daily physical examination, and roentgenogram of the chest.

The antifibrinolysin response in hospitalized soldiers with presumptive streptococcal infections is shown in Table III. Included in the table are 232 patients who were found to have exudate on the tonsils or pharynx and from whom the β-hemolytic streptococcus was isolated during the first 3 days of hospitalization. There were 151 in the group, or 65 per cent, who not only had clinical and bacteriological evidence of strep-

TABLE III

Antifibrinolysin and antistreptolysin response in 232 patients with exudative tonsillitis or pharyngitis from whom β-hemolytic streptococci were isolated

Antistreptolysin response	Antifibrinolysin response		Totals	Antifibrinolysin response
	number positive	number negative		per cent positive
Number positive	56	95	151	37
Number negative	12	69	81	15
Totals	68	164	232	29

Definition: A difference in titer of antistreptolysin or antifibrinolysin of two succesive tube dilutions between the acute-phase and convalescent-phase sera was considered a positive test.

tococcal infection, but also responded by an increase in antistreptolysin antibodies. This group of 151 men was considered to have had *proved* infections caused by the β-hemolytic streptococcus. The antifibrinolysin titer was found to increase significantly in only 56, or 37 per cent of these patients. This indicated that the antifibrinolysin response was not as sensitive an index of streptococcal infection as the antistreptolysin response.

Since it has been demonstrated that antistreptolysin antibodies increase significantly in only 85 per cent of probable streptococcal infections, scarlet fever (10) and epidemic foodborne septic sore throat (13), it was of considerable importance to determine the frequency with which antifibrinolysin responses occurred in the absence of an increase in antistreptolysin antibodies. There were 81 patients with exudative tonsillitis harboring β-hemolytic streptococci who failed to respond to the infection by an increase in antistreptolysin titer. Twelve of these patients, or 15 per cent, exhibited an increase in antifibrinolysin antibodies.

Significant increases in both antifibrinolysin and antistreptolysin antibodies were less frequent in subjects with respiratory disease from whom β-hemolytic streptococci were isolated, but in whom no exudate was present on the lymphoid tissues of the throat. In 140 such patients (Table IV), 41, or 27 per cent, exhibited an increase in antistreptolysin antibodies, and 21, or 15 per cent, developed antifibrinolysin antibodies. Among patients without an antistreptolysin response, only 7 per cent developed an increase in antifibrinolysin antibodies.

TABLE IV

Antifibrinolysin and antistreptolysin response in 140 patients with respiratory disease other than exudative tonsillitis and from whom β-hemolytic streptococci were isolated

Antistreptolysin response	Antifibrinolysin response		Totals	Anti-fibrinolysin response
	number positive	number negative		per cent positive
Number positive	14	27	41	34
Number negative	7	92	99	7
Totals	21	79	140	15

Specificity of the antifibrinolysin response

The data presented in Tables III and IV, show, therefore, that antifibrinolysin antibodies increased following clinical infection with the β-hemolytic streptococcus. Further studies of the specificity of the reaction were then carried out.

Of 404 well soldiers, antifibrinolysin and antistreptolysin antibodies were determined on sera collected from 390. The change in titer between the initial serum specimen and a subsequent sample collected 21 to 60 days later is presented in Table V according to the presence or absence of β-hemo-

TABLE V

Antifibrinolysin response in 390 well soldiers according to the presence or absence of β-hemolytic streptococcus in the oropharynx and the antistreptolysin response

Antistreptolysin response	β-hemolytic streptococcus			
	Present		Absent	
	Antifibrinolysin response		Antifibrinolysin response	
	number positive	number negative	number positive	number negative
Number positive	0	17	0	0
Number negative	1	136	0	236

lytic streptococci during the period of study. Those subjects who never carried β-hemolytic streptococci did not show an increase in either antibody. Seventeen of the men who were carriers of the β-hemolytic streptococcus showed an increase in antistreptolysin antibodies and one developed antifibrinolysin antibodies. These data demonstrate that an increase in titer of either antibody in the absence of β-hemolytic streptococci was not observed. Carriers, however, may occasionally show an increase in antistreptolysin

antibodies but rarely an increase in antifibrinolysin titer.

The antibody response to non-specific infections was studied in 244 sets of sera obtained from soldiers hospitalized for respiratory disease. The diagnoses included 200 cases of acute undifferentiated respiratory disease, 19 patients with atypical pneumonia, and 25 cases of a miscellaneous group including penumococcal pneumonia, pulmonary tuberculosis, sinusitis, and influenza A. No instance of exudative tonsillitis or pharyngitis was included, nor in any case, were β-hemolytic streptococci isolated from the throat during the first 3 days of hospitalization.

Of the 244 sets of sera examined, 232 convalescent sera did not exhibit an increase in either antifibrinolysin or antistreptolysin antibodies (Table VI). In 12 patients the antistreptolysin titer of the convalescent serum was significantly increased over the acute phase specimen. In 3 of these patients there was also an increase in antifibrinolysin antibodies. These results demonstrated, therefore, the high degree of specificity of the antifibrinolysin test, in that in only 3 instances was an increase

TABLE VI

Antifibrinolysin and antistreptolysin response in 244 hospitalized soldiers with respiratory disease other than exudative tonsillitis and from whom no β-hemolytic streptococci were isolated

Antistreptolysin response	Antifibrinolysin response		Totals	Anti-fibrinolysin response
	number positive	number negative		per cent positive
Number positive	3	9	12	25
Number negative	0	232	232	0

in antibodies demonstrated in the absence of clinical and bacteriological evidence of streptococcal infection, and in each of the 3 sets of sera a concomitant increase in antistreptolysin titer was observed.

The question of the specificity of the antistreptolysin response observed in this group of patients cannot be answered. It is to be emphasized, however, that bacteriological data were obtained only during the first 3 days of hospitalization, so that hospital cross infection, or inapparent infections in the field may have occurred prior to the time the convalescent blood was taken.

The only instances of an antifibrinolysin re-

sponse in the absence of either bacteriological or serological confirmation of the streptococcus etiology occurred in a group of 115 soldiers hospitalized for exudative tonsillitis from whom no β-hemolytic streptococci were isolated. In 4 of these patients the titer of antifibrinolysin increased during convalescence without a concomitant increase in the antistreptolysin titer. The sera from these 4 patients had been stored 16 months prior to the determination, and they were known to be contaminated. Whether they represent true instances of non-specific antifibrinolysin reactions cannot be determined.

DISCUSSION

According to the review of Tillett (8) 85 to 90 per cent of plasma collected from normal individuals was found to contain little or no antifibrinolysin as indicated by the lysis of such plasma clots within one hour. The results obtained by different investigators (8) are in general agreement, in spite of the fact that the amount or source of fibrinolysin used was not standardized. In the present study the fibrinolysin was obtained from a group A strain of β-hemolytic streptococcus, and in all tests a standard unit of fibrinolysin was employed. Under these conditions a titer of 50 units or less was exhibited by 66 per cent of sera collected from normal subjects, indicating the presence of little or no antifibrinolysin. In approximately 11 per cent of the normal subjects the antifibrinolysin antibodies were considered to be elevated in that the titer was greater than 150 units. Normal individuals found to harbor group A β-hemolytic streptococci in the oropharynx showed a slightly different distribution of the antifibrinolysin titers with 15 per cent greater than 150 units.

That the high antifibrinolysin titers observed in normal subjects resulted from previous experience with the β-hemolytic streptococcus was indicated by the fact that the antistreptolysin titer was usually elevated in those sera which also showed a high antifibrinolysin titer. However, it was also noted that elevated antistreptolysin titers are not always associated with a high antifibrinolysin titer, in fact, in only 20 per cent of sera whose antistreptolysin titer was 159 or greater was the antifibrinolysin titer greater than 150 units. These results would indicate then, that streptococcal infections

produce an antistreptolysin response more frequently than an antifibrinolysin response. This was confirmed when the antibody response was studied in 232 hospitalized soldiers with exudative tonsillitis or pharyngitis from whom β-hemolytic streptococci were isolated from the throat cultures. Of this group, 151 showed an increase in antistreptolysin antibodies, whereas in only 68 was there an increase in the antifibrinolysin titer. As a routine diagnostic test, therefore, the demonstration of an increase in antistreptolysin titer is a more sensitive index of streptococcal infection than an increase in antifibrinolysin antibodies.

Since not all patients with streptococcal infections exhibit an increase in antistreptolysin during convalescence, the additional determination of antifibrinolysin antibodies has proved valuable. Approximately 15 to 20 per cent of patients with scarlet fever (10) or epidemic exudative tonsillitis (13) fail to develop antistreptolysin during the convalescent period, and yet there is little doubt that the infection was caused by the β-hemolytic streptococcus. In a recent study of a food-borne epidemic of exudative tonsillitis and pharyngitis caused by a type 5 β-hemolytic streptococcus, 80 of 100 patients showed a significant antistreptolysin response (13). Of the entire group, 20 showed an increase in antifibrinolysin antibodies, 4 of which occurred in patients who showed no significant change in the antistreptolysin titer. The serological response in 232 soldiers with exudative tonsillitis from whom β-hemolytic streptococci were isolated show that 12 individuals, or 5 per cent, exhibited an increase in antifibrinolysin titer without a concomitant increase in antistreptolysin.

The data in the present report demonstrate that an increase in titer of two dilution increments during convalescence is highly specific. A total of 98 individuals showed an increase in antifibrinolysin antibodies, in 70 of whom there was both bacteriological and serological (antistreptolysin) evidence of streptococcal infection. In 20 there was only bacteriological evidence, and in 4 only serological indication of the streptococcal etiology. In 4 cases of exudative tonsillitis the development of antifibrinolysin antibodies was the only evidence of infection caused by the β-hemolytic streptococcus.

In contrast to the studies reviewed by Tillett (8) which showed that 67 per cent of patients with

streptococcal tonsillitis develop antifibrinolysin antibodies during convalescence, is the fact that only 37 per cent of the present series of patients with streptococcal infections of the throat, as proved by an increase of antistreptolysin, showed an increase in the antifibrinolysin titer. The cause for these differences was not at once apparent. The lack of adequate convalescent serum specimens does not explain the low incidence of antifibrinolytic responses observed in this study, since one or more sera obtained 3 to 6 weeks after the initial bleedings were tested in almost every instance. Although the majority of the patients with *proved* streptococcal infections reported in this study were only mildly ill, there were no obvious differences in the severity of illness of those patients who did or did not exhibit an increase in the antifibrinolytic titer.

Recently it has been demonstrated that the different types of streptococci vary in their capacity to produce erythrogenic toxin as measured by the incidence of scarlet fever (14). Similarly, some evidence was obtained in this laboratory that different strains of streptococci varied in their ability to stimulate antistreptolysin formation (12). These observations suggested that β-hemolytic streptococci may vary in their ability to produce an antifibrinolytic response.

Extensive clinical and laboratory studies were therefore undertaken to determine the factors which are responsible for the stimulation or lack of stimulation of antifibrinolysin. A preliminary report of these investigations presented elsewhere (5) demonstrated that the increase in antifibrinolysin titer during convalescence was dependent on the strain of group A streptococcus responsible for the infection. Endemic infections caused by type 3 streptococci resulted in few antifibrinolytic responses (10 per cent), whereas 62 per cent of infections due to type 19 showed an increase in antifibrinolysin. Likewise, types 5 and 12 streptococci producing epidemic sore throat showed marked differences in their ability to stimulate antibody formation, with 20 and 92 per cent respectively of the patients exhibiting an increase in the antifibrinolysin titer. The amount of fibrinolysin produced by these streptococci was measured, and it was found that, in general, organisms which stimulate antifibrinolysin formation *in vivo*,

produced large amounts *in vitro*, while those streptococci that produced small amounts of fibrinolysin generally failed to stimulate antibody formation.

SUMMARY

Quantitative antifibrinolysin estimations were made of sera collected from 1204 subjects. Analysis of the data thus obtained showed that sera from the majority of normal subjects contain little antifibrinolysin. In approximately 12 per cent of normal individuals, the titer was elevated above 150 units, presumably due to previous experience with the β-hemolytic streptococcus.

An increase in the antifibrinolysin titer of two dilution increments was shown to be indicative of a previous infection by the β-hemolytic streptococcus. Antifibrinolysin developed in 29 per cent of patients with exudative tonsillitis from whom β-hemolytic streptococci were cultured, whereas antistreptolysin increased in 65 per cent.

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EFFECTS OF THYROID ON CREATINE METABOLISM WITH A DISCUSSION OF THE MECHANISM OF STORAGE AND EXCRETION OF CREATINE BODIES [1]

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The vast literature which has accumulated on the metabolism of creatine has been reviewed in a number of monographs (1 to 4). The discovery of phosphocreatine in muscle (5, 6) was followed by extensive work on the enzyme system controlling the creatine-phosphocreatine cycle and the rôle of this cycle in muscular contraction (7, 8). The synthesis of creatine from its precursors has recently been elucidated using isotopes (9, 10) and experiments on isolated tissues (11). It has been demonstrated that administration of androgens having a methyl group in the 17-position leads to an outpouring of creatine into the urine, presumably through an increased synthesis of creatine (12 to 14).

In spite of all this work, the mechanism by which various physiological and pathological conditions influence the excretion of creatine and creatinine is still little understood. The elucidation of these problems depends on the one hand on the study of the synthesis of creatine from its precursors, and on the other hand, upon an understanding of the factors which govern the storage of creatine and phosphocreatine in the muscles, and the liberation and excretion of creatine and creatinine in the urine. The effects of thyroid on the excretion of creatine are so marked that they present an opportunity to study these questions. In this paper we shall survey briefly knowledge which has accumulated on the relationship of thyroid to creatine metabolism and add to it observations which we have made. We shall then discuss the bearing of these findings upon the mechanism of the storage and excretion of creatine and creatinine.

Excretion of creatine and creatinine in hyperthyroidism and hypothyroidism

The relation of thyroid to the excretion of creatine was first pointed out in 1908 by Shaffer (15) who found increased creatine and decreased creatinine excretion in Graves disease. It is now generally recognized that the output of creatine is increased in hyperthyroidism (16 to 18), and that the physiological creatinuria of childhood is absent or decreased in hypothyroid children (19 to 23, 31).

Less attention has been paid to the effect of thyroid on the output of creatinine since the changes involve a relatively small percentage of the total output of this substance. Some writers consider these changes as insignificant (16). However, the original observation of Shaffer that the output of creatinine is markedly decreased in hyperthyroidism has been confirmed by others (18, 24). Wang (4) found an average daily urine creatinine excretion of 11.7 mgm. per kgm. in females with thyrotoxicosis, compared to a normal range of 14 to 22 mgm. per kgm. He did not demonstrate any alteration in the creatinine output in two cases of myxedema studied.

Although the total output of combined creatine + creatinine is of great importance in the study of the mechanism of storage and excretion, most writers have paid little attention to this. Eimer (25) reported total daily creatine + creatinine excretion of 11.6 to 17.7 mgm. per kgm. in women suffering from Graves disease, as compared to an excretion of from 18 to 22 mgm. per kgm. in normal women. Our own observations agree with those of most workers. However, a comparison of the coefficients of creatinine and of total creatine + creatinine in groups of hyperthyroid and hypothyroid patients with those of normal patients, does not give a true comparison of the output of such patients in relation to the actual muscle mass. The coefficients based on total body weight may be

misleading, since in the thin hyperthyroid patient the relative amount of muscle may be increased due to a depletion of fat, and in the hypothyroid patient the proportion of muscle is decreased because of the large amount of myxedematous fluid. More reliable information may be obtained by studying the excretion of a hyperthyroid or a hypothyroid patient before treatment and again after he has been adjusted through treatment to a comparatively normal state. We shall present such data in subsequent sections, but our general conclusions on the subject may be presented at this point.

In relation to the normal, the *creatine* output is increased in hyperthyroidism, decreased in hypothyroidism.

The *creatinine* output is decreased in hyperthyroidism and increased in hypothyroidism.

The *total creatine + creatinine* is relatively little affected; in hyperthyroidism there may be a significant decrease in some cases; in hypothyroidism the total output differs little from the normal but may be slightly increased.

Muscle creatine and phosphocreatine in hyperthyroidism and hypothyroidism

In thyrotoxic rats a significant decrease of the creatine content of the striated muscle (26) and a decrease of both phosphocreatine and creatine in heart muscle (27) has been demonstrated. Wang (4), using improved methods, studied both creatine and phosphocreatine in rabbits. His results are shown in Table I.

Although the statistical significance of Wang's data becomes evident only if the thyroxinized and thyroidectomized rabbits are compared, his observations suggest that both creatine and phosphocreatine are decreased in hyperthyroidism and increased in hypothyroidism. It is probable that

TABLE I

Effects of thyroid on muscle creatine of rabbits (Wang)

	No. of animals	Free creatine	Phospho-creatine	Total creatine
		mgm. per 100 grams of muscle		
Normal rabbits	12	211±25	247±18	458±26
Thyrotoxic rabbits	12	205±33	210±13	415±30
Thyroidectomized rabbits	5	237±30	269±11	505±25

the differences would have been more striking if allowance had been made for the fact that muscle tissues retain fluid in myxedema and are depleted of fluid and fat in hyperthyroidism.

Treatment of thyrotoxic patients with iodine or with thiouracil

Wang studied 23 females with thyrotoxicosis who were treated with iodine for periods of 15 to 55 days. He found in most instances a decrease in the creatine output, but states that the creatinine excretion was not altered. In reviewing these cases it appears that most did not have complete remission with iodine, the B.M.R. remaining + 20 to + 85 per cent. If we select the three cases studied after operation (No. 48 to 50), and one treated with iodine with a reduction of the B.M.R. to + 10 per cent (No. 59), we note the following results (Table II).

In these four cases the creatinine output increased and the creatine diminished as the thyrotoxicosis improved. In two of the cases the total creatine + creatinine output decreased and in the other two it increased. A survey of the data reported in the literature shows that in thyrotoxic patients treated with iodine (16, 28) or with thiouracil (29, 30) the rise in creatinine excretion is not as consistent as the drop in creatine excretion.

TABLE II

Effect of treatment on the creatine-creatinine excretion of thyrotoxic patients (Wang)

	Before treatment (first 3 days)				After treatment				
	B.M.R.	Creatine	Creatinine	Total	Treatment	B.M.R.	Creatine	Creatinine	Total
	per cent	mgm.	mgm.	mgm.		per cent	mgm.	mgm.	mgm.
Case No. 48	+62	547	514	1061	Operation	+34	85	552	637
Case No. 49	+47	475	732	1207	Operation	+39	52	803	855
Case No. 50	+50	152	735	887	Operation	−5	2	832	834
Case No. 59	+52	117	647	761	Iodine	+10	30	957	987
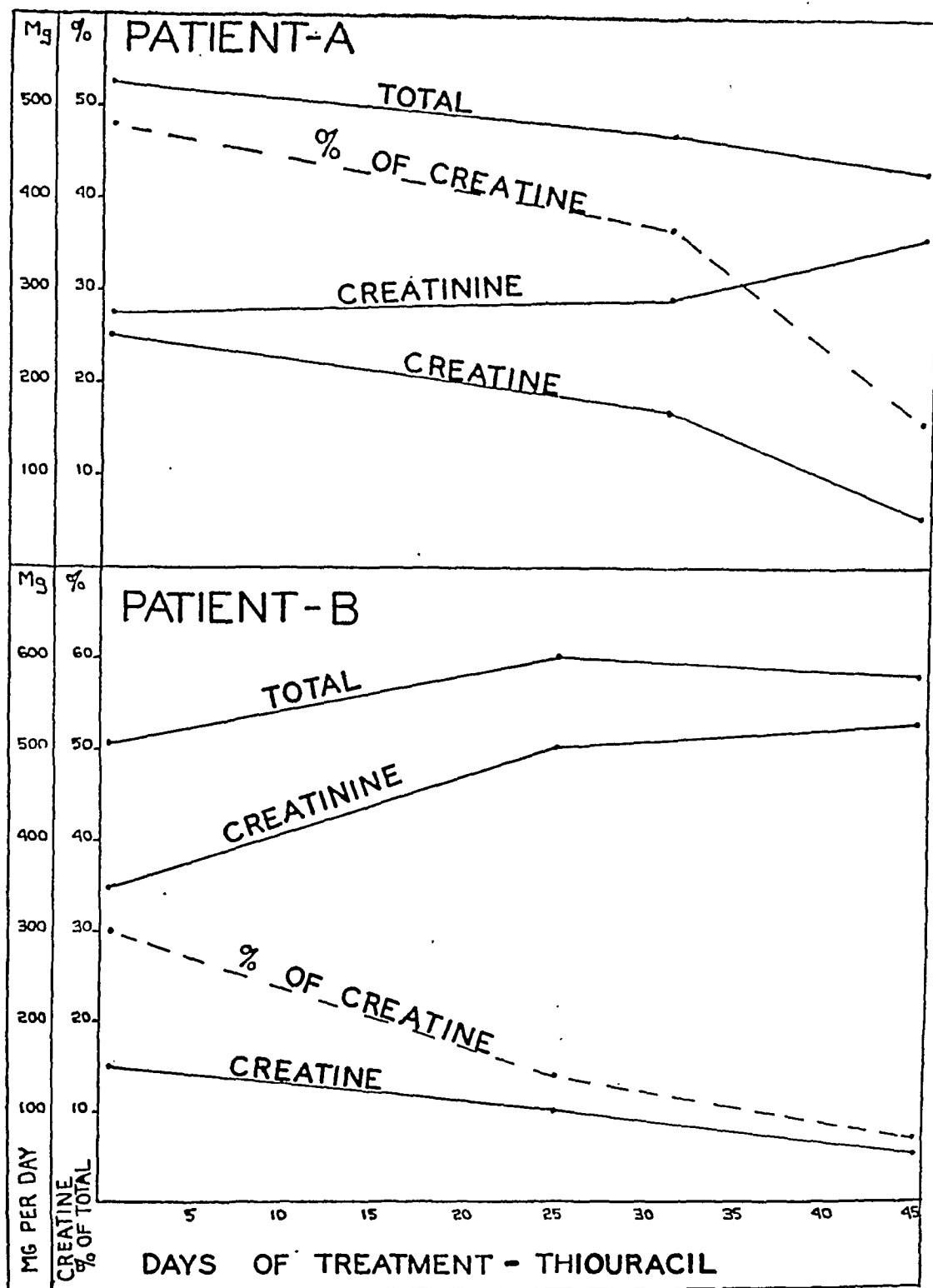
FIG. 1. TREATMENT OF THYROTOXICOSIS WITH THIOURACIL

				B.M.R.	Cholesterol
Patient A:	Jesse H.	11 yrs.	Before treatment	+ 43 to + 47 per cent	155 mgm. per cent
			After treatment	+ 44 to + 45	130
Patient B:	Joyce G.	13 yrs.	Before treatment	+ 24 to + 35	121–130
			After treatment	+ 7 to 0	132–150

The line designated "per cent of creatine" indicates the ratio of creatine: creatine + creatinine.

Our own experience with thiouracil therapy in children has been limited to two cases. In both of these cases when a creatine-free diet was given treatment caused a decrease in the output of creatine and an increase of creatinine. The total creatine + creatinine was decreased in one case and increased slightly in the other. These results are illustrated graphically in Figure 1.

Treatment of hypothyroidism with desiccated thyroid

The effects of thyroid are shown most clearly when an athyrotic patient is first given thyroid therapy and the output of creatine and creatinine is measured daily for periods of 40 to 60 days. In previous papers we published data on the treatment of hypothyroid children (31) and subsequently we
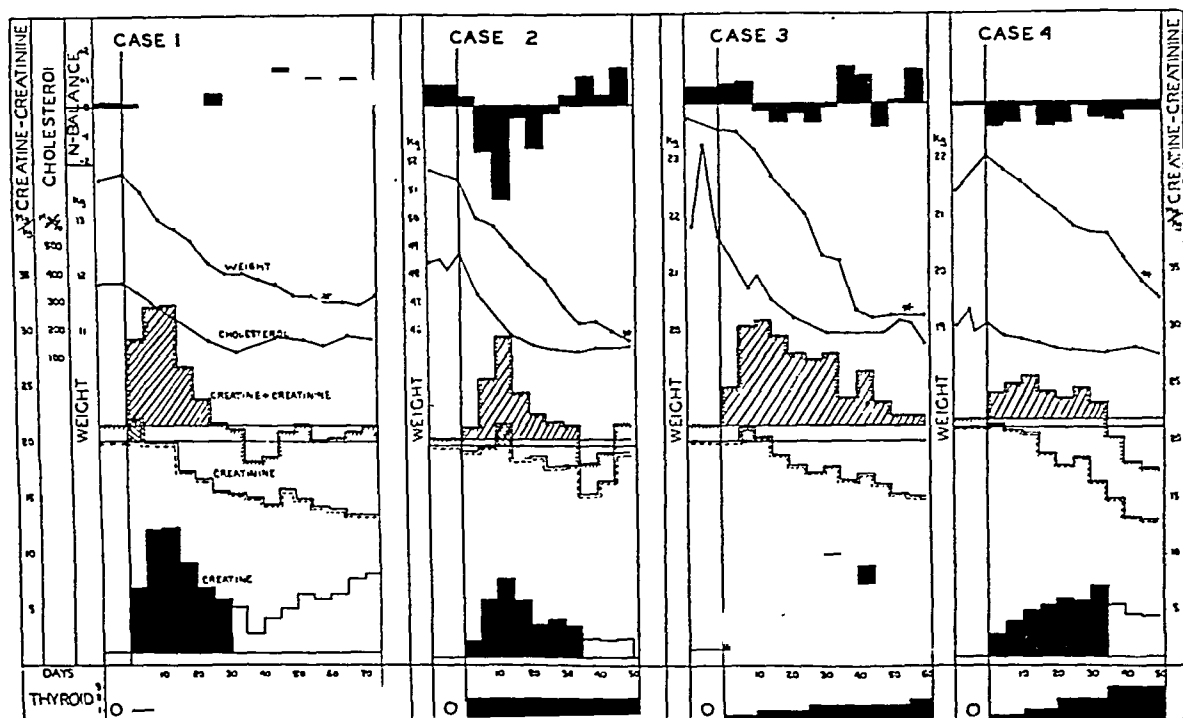
FIG. 2. TREATMENT OF HYPOTHYROID PATIENTS WITH THYROID

Case 1. Ronnie Z. Age 4 yrs. 4 mos. Bone age 3 mos. Height-age 1½ yrs. Previously untreated.

Case 2. Gertrude S. Age 27 yrs. Cretin—mentally defective. Untreated until she was 22 yrs., then treated intermittently. No treatment for 6 months prior to study.

Case 3. Price R. Age 14 yrs. Bone age 5 yrs. Height-age 5¾ yrs. Previously untreated.

Case 4. Samuel A. Age 10½ yrs. Bone age 3 yrs. Height-age 4¾ yrs. Previously untreated.

In each case the output of creatine, creatinine and total is shown for an average of 10 days without treatment. The subsequent changes in excretion under treatment are shown as averages of 5-day periods. During the first phase of treatment the excretion of creatine + creatinine was elevated above the pretreatment level. The total creatine + creatinine lost is indicated by the shaded areas. The increase in the output of creatine during the corresponding period is shown by the black area. Following the first phase of treatment the output of creatine + creatinine was reduced to or slightly below the pretreatment level, but the creatine was relatively increased and the creatinine decreased. The nitrogen balances shown at the top of the charts indicate that there were small deficits during the first phase of treatment.

* Indicates weight used in calculating creatine and creatinine coefficients throughout experiment. The weight was selected at the end of the treatment period because this gives a better index of the tissue mass than the weight before or during treatment. The reason for this is that there are excess fluid stores in the myxedematous state. The marked decrease in weight during thyroid treatment is due almost entirely to the loss of body fluids and not to the loss of tissue proteins. Nitrogen balance studies carried out simultaneously showed that during the first 20 to 30 days of treatment there was only a slight loss of body protein and that after this there was a positive N-balance.
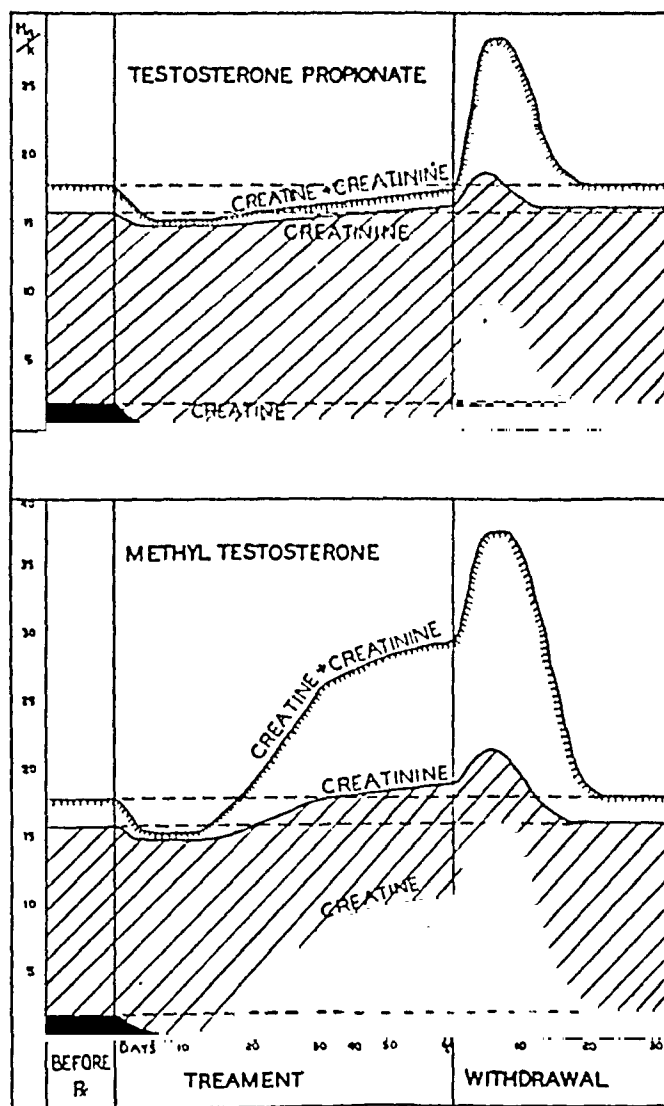
FIG. 3. EFFECTS OF TESTOSTERONE PROPIONATE AND
METHYL TESTOSTERONE ON PATIENTS WHO ARE NOT
HYPOTHYROID

These curves represent averages. There are wide vari-
ations of the levels in individual cases (13).

contrasted the type of curve observed with that
found when patients are treated with methyl
testosterone (13). (Compare Figures 2 and 3).
Other workers (32, 21) recorded similar effects
from thyroid medication.

In Figure 2 we have recorded new observations
made on four hypothyroid patients receiving diets
containing less than 30 mgm. of creatine daily dur-
ing the first 50 to 70 days of thyroid medication.
The essential similarity of these charts is apparent
in spite of minor variations which may depend
upon the intensity of treatment and differences in
the patients. The effects of treatment are perhaps
illustrated best in cases 1 and 2, for which data
are given in Table III.

In case 1 the output of total *creatine + creati-
nine* increased abruptly on the administration of
thyroid, rising from a level of 21.6 mgm. per kgm.
to a peak of 32.3 mgm. per kgm. between the 6th
and 15th days. The excretion then decreased un-
til it reached the pretreatment level by the 30th
day. After this it remained at or slightly below
the level which had existed during the hypothyroid
state in spite of subsequent increase in the dose of
thyroid.

The excretion of *creatine* rose from 1.3 mgm.
per kgm. to a peak of 12.3 mgm. per kgm. be-
tween the 6th and 15th day. By the 30th day it
had decreased to 5.2 mgm. per kgm. After this

TABLE III
Effect of treatment of hypothyroid patients

Days of treatment	Creatinine		Creatine		Total		Ratio Creatine/ Total	Thyroid dose
	mgm.	mgm. per kgm.*	mgm.	mgm. per kgm.	mgm.	mgm. per kgm.	per cent	mgm.

Case 1

Days of treatment	mgm.	mgm. per kgm.*	mgm.	mgm. per kgm.	mgm.	mgm. per kgm.	per cent	mgm.
Control	233	20.3	15	1.3	248	21.6	6	0
1 to 5	255	22.2	79	7.0	334	29.2	24	64
6 to 10	231	20.0	139	12.2	370	32.2	38	64
11 to 15	230	20.0	141	12.3	371	32.3	38	64
16 to 20	201	17.5	106	9.2	307	26.7	34	64
21 to 25	193	16.8	82	7.1	275	23.9	30	64
26 to 30	182	15.8	69	6.0	251	21.8	27	64
31 to 35	179	15.8	59	5.2	238	20.7	25	64
36 to 40	176	15.3	33	2.9	209	18.2	16	64
41 to 45	167	14.5	50	4.3	217	18.8	21	64
46 to 50	183	15.9	60	5.2	243	21.1	25	128
51 to 55	173	15.0	74	6.5	247	21.5	30	128
56 to 60	164	14.3	71	6.1	235	20.4	30	192
61 to 65	163	14.2	74	6.4	237	20.6	31	192
66 to 70	156	13.5	86	7.5	242	21.0	36	192

Case 2

Days of treatment	mgm.	mgm. per kgm.*	mgm.	mgm. per kgm.	mgm.	mgm. per kgm.	per cent	mgm.
Control	885	19.8	37	0.7	922	20.5	4	0
1 to 5	861	19.1	98	2.2	959	21.3	10	128
6 to 10	882	19.6	273	6.1	1155	25.7	24	128
11 to 15	974	21.6	352	7.9	1326	29.5	27	128
16 to 20	831	18.5	266	5.9	1097	24.4	24	128
21 to 25	848	18.8	168	3.8	1016	22.6	17	128
26 to 30	805	17.9	181	4.2	986	21.9	19	128
31 to 35	811	18.0	169	3.6	980	21.6	17	128
36 to 40	693	15.4	122	2.7	815	18.1	15	128
41 to 45	747	16.6	112	2.5	859	19.1	13	128
46 to 50	868	19.1	111	2.6	979	21.7	12	128

* In case 1—the coefficients were calculated for weight of 11.5 kgm. throughout experiment.
In case 2—calculations were for weight of 45.0 kgm.

the output fluctuated between 2.9 and 5.2 mgm. per kgm. during treatment with 64 mgm. of thyroid. When the dosage of thyroid was increased progressively up to 192 mgm. daily, the creatine output increased up to 7.5 mgm. per kgm.

The excretion of *creatinine* showed decided changes of a different sort. The ouput was slightly above the ·pretreatment level during the first 5 days. After the 15th day it decreased progressively reaching a level of 13.5 mgm. per kgm. compared to 20.3 mgm. per kgm. before treatment.

It is apparent that the effects of thyroid treatment fall into two phases. In the *first phase* (in this case lasting 30 days) there was an output of total creatine + creatinine above the hypothyroid

level. In the *second phase,* the total excretion was approximately at the same level as during the hypothyroid state or perhaps slightly below it. During this phase, however, the creatine excretion was considerably increased and the creatinine excretion decreased in relation to the hypothyroid levels. Before treatment, free creatine constituted 6 per cent of the total; after adjustment to a dose of 64 mgm. of thyroid, 16 to 21 per cent. Increasing the dose of thyroid up to 192 mgm. caused the proportion of creatine to increase to 36 per cent, although the total output of creatine + creatinine was not affected.

Although the most obvious explanation of the temporary increase in the total creatine + creatinine output which occurs during the first phase of treatment, is an outpouring of stores of creatine from the muscles, it is necessary to consider other possible explanations. One other explanation might be that the patient loses his original sensitivity to thyroid and fails to respond after a time. This is unlikely, because in all cases the serum cholesterol remained at a low level, the B.M.R. continued to rise and clinical improvement progressed. The weight did not increase after the total creatine + creatinine excretion returned to the hypothyroid level. Another explanation might be that thyroid temporarily causes an increased synthesis of creatine. We shall disprove this in our further discussion by observations indicating that thyroid does not influence the synthesis of creatine caused by methyl testosterone. If then we admit that there is a release of creatine stores during the first stage of treatment, the amount released can be calculated in case 1 as follows:

	Total creatine + creatinine mgm.	Creatine mgm.	Creatinine mgm.
Excretion during treatment days 1 to 30	9540	3080	6460
Excretion at pretreatment level for 30 days	7440	450	6990
Difference	+2100	+2630	− 530

The total creatine + creatinine lost from the body amounted to 2100 mgm. However, the creatine output was 2630 mgm. greater than would have occurred at the hypothyroid level of excretion. The difference of 530 mgm. is accounted for by a corresponding decrease in the output of creatinine.

The amounts of stores lost from the body (shown in Figure 2 by the black or shaded areas) vary in different cases and are not in exact relation to the patient's weight. It is apparent that in each case the increase in the amount of creatine put out during this period was greater than that of total creatine + creatinine. This means that a portion of the creatinine output comes directly from the stores and the remainder is due to a decrease in the conversion of creatine into creatinine.

The effect of testosterone propionate and methyl testosterone on the creatine metabolism of normal and hypogonadal patients

In a previous paper (13) we have discussed the effects of androgens on creatine metabolism. As shown in Figure 3, testosterone propionate causes a decreased excretion of creatine and creatinine. This steroid brings about a rapid growth of muscle tissue for which creatine and phosphocreatine undoubtedly are needed. The decreased excretion
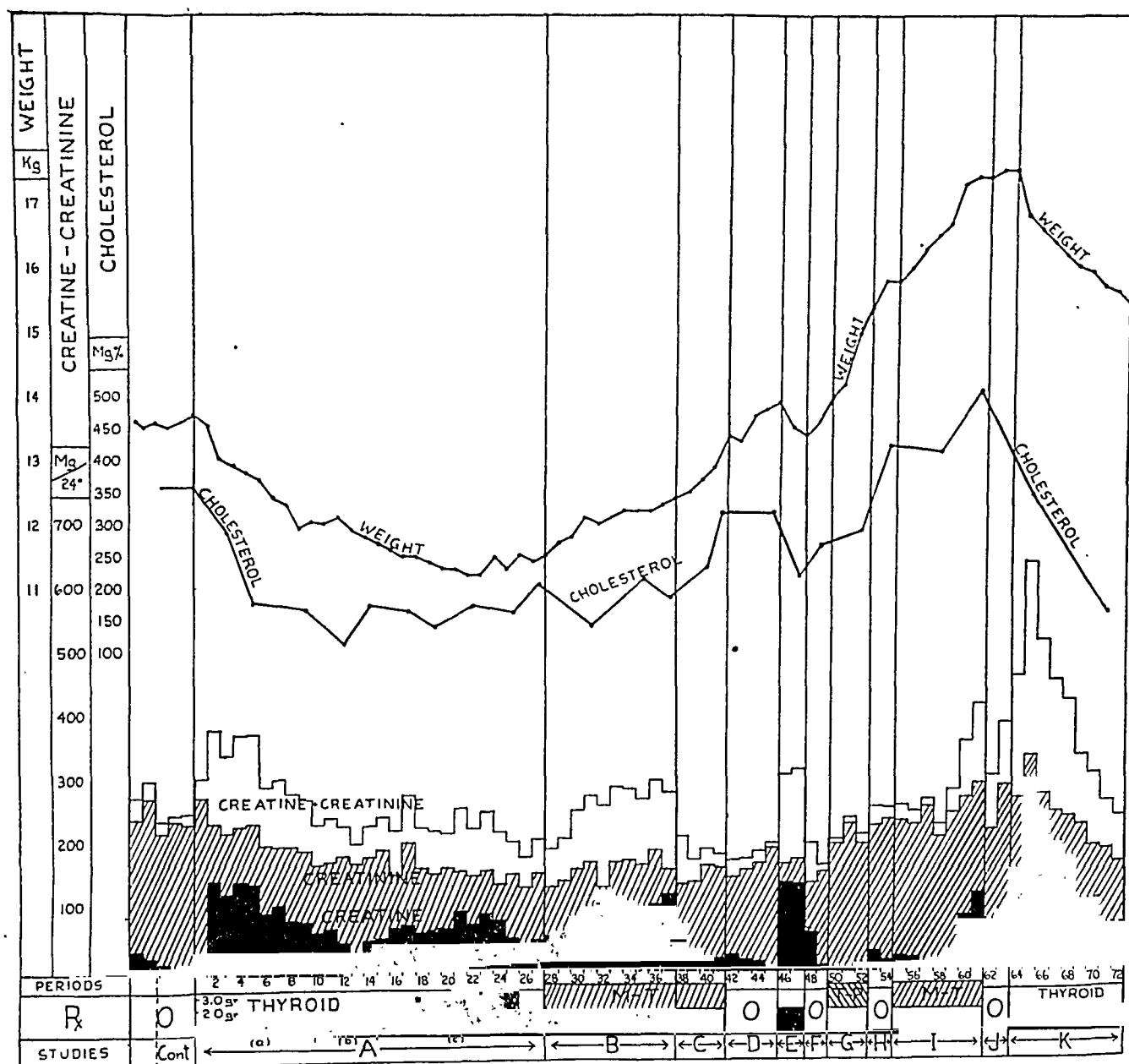
FIG. 4. CASE 1. HYPOTHYROID PATIENT, AGED 4½ YEARS. EFFECTS OF THYROID, METHYL TESTOSTERONE, TESTOSTERONE PROPIONATE AND COMBINATIONS

Each column represents an average of a 5-day period
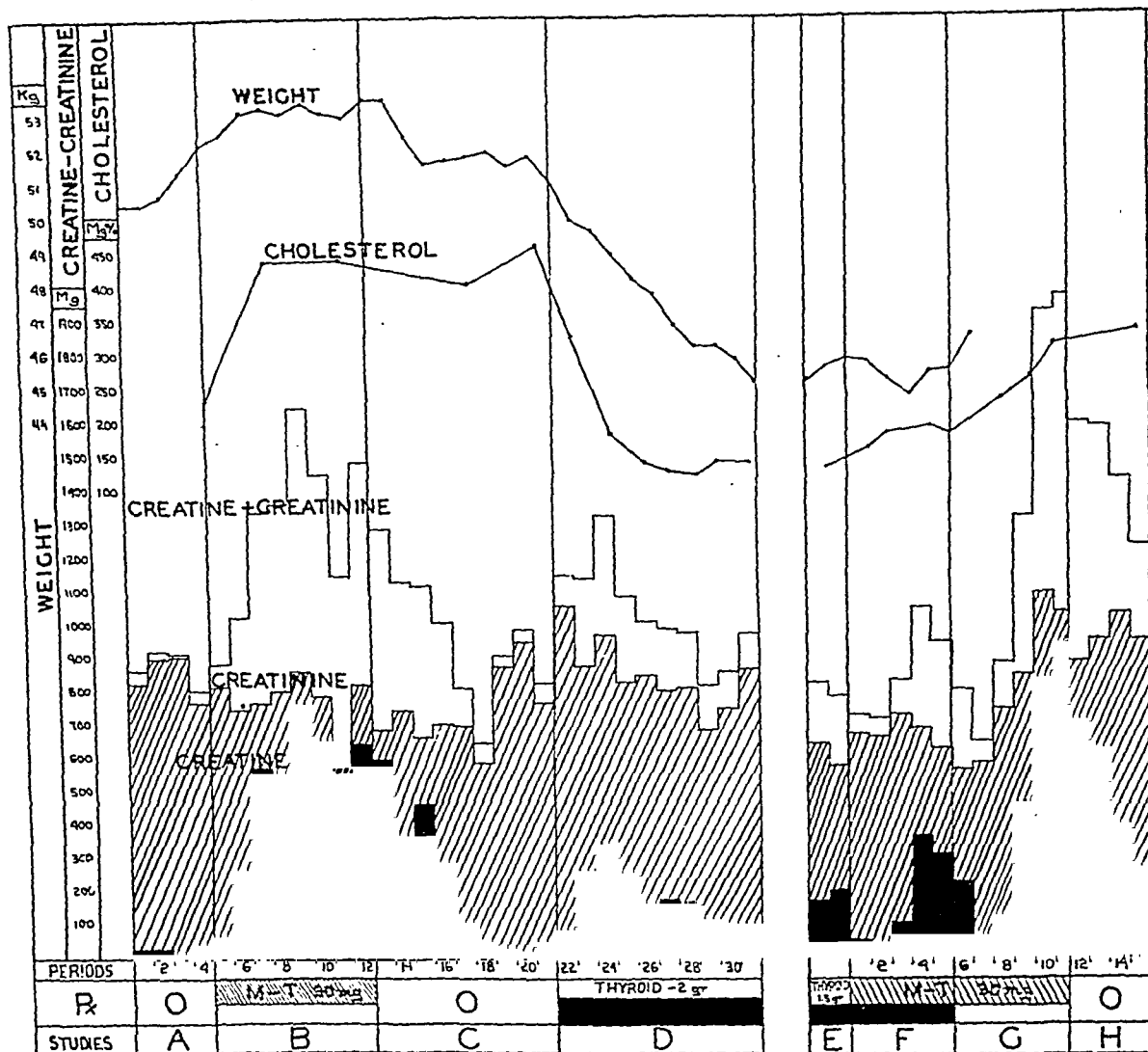
FIG. 5. CASE 2. HYPOTHYROID PATIENT, AGED 27 YEARS. EFFECTS OF THYROID PLUS METHYL TESTOSTERONE AND COMBINATIONS

Each column represents an average of a 5-day period

may be due to the fact that the increased needs of the body for creatine are not fulfilled by a corresponding increase of synthesis. The temporary loss of creatine and creatinine which occurs on discontinuing testosterone propionate resembles closely the negative nitrogen balance which has been observed on withdrawing androgenic therapy (34). Apparently when the stimulus to increased anabolism is withdrawn, a portion of the retained nitrogen which may be loosely bound is lost. It is possible that a portion of the creatine and phosphocreatine also is more loosely bound than the rest and is readily lost.

Methyl testosterone has a two-fold effect on creatine metabolism. It increases the need for creatine and phosphocreatine in building new muscle in the same way as does testosterone propionate, thus causing a slight temporary decrease in the excretion of creatine and creatinine. However, it also causes a marked increase in the rate of synthesis of creatine from its precursors, so that after 10 to 20 days the demands are exceeded, and increasing amounts appear in the urine. Discontinuing methyl testosterone causes a release of part of the stores as in the case of testosterone propionate.
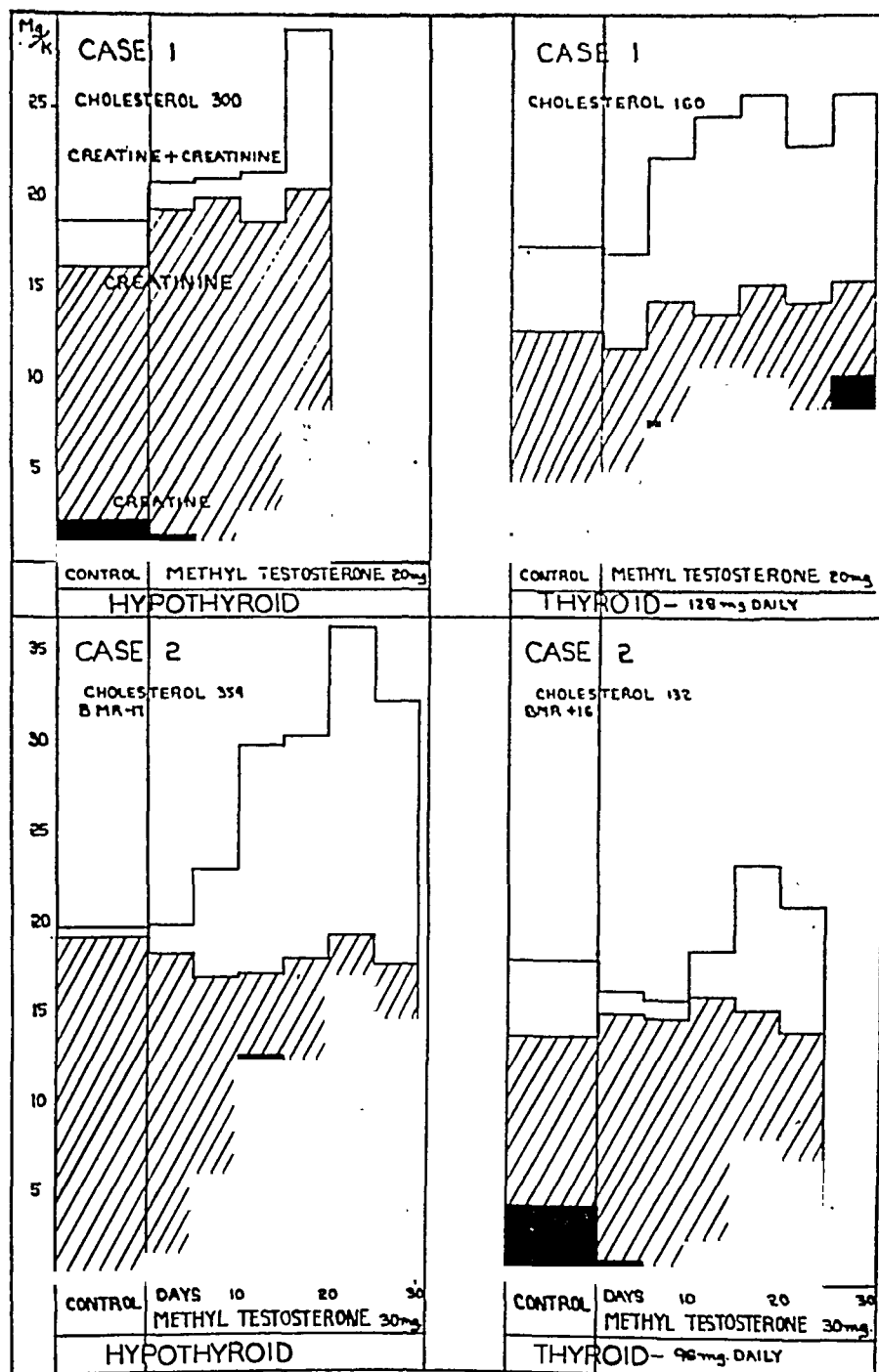
FIG. 6. EFFECTS OF METHYL TESTOSTERONE IN HYPOTHYROID PATIENTS
WHEN UNTREATED AND WHEN CORRECTED WITH THYROID

Each column represents an average of a 5-day period.

Each of these hypothyroid patients was tested with methyl testosterone while untreated with thyroid and having a high serum cholesterol, and again when well adjusted on thyroid therapy and having a low serum cholesterol. In each case, whether treated or untreated, the excretion of creatine and creatine + creatinine increased although there were variations in the rapidity of the response.

A number of experiments with methyl testosterone were made on two hypothyroid patients receiving a creatine-free diet, both while they were in the hypothyroid stage and while they were adjusted to an apparently normal condition by thyroid therapy. *Patient 1* was a hypothyroid dwarfed boy of 4 years and 4 months who had a bone age of 3 months, and had never been treated previously. *Patient 2* was a female cretin of 27 years who had been untreated until she was 22 years and was then treated intermittently. She was allowed to lapse into a hypothyroid state preparatory to the present studies. The complete studies are shown in Figures 4 and 5. Several phases of these studies need special emphasis.

In Figure 6 are shown the effects of methyl testosterone when given to these two patients in the hypothyroid state in comparison with the effects exhibited by this steroid when these patients were adjusted to a comparatively normal condition
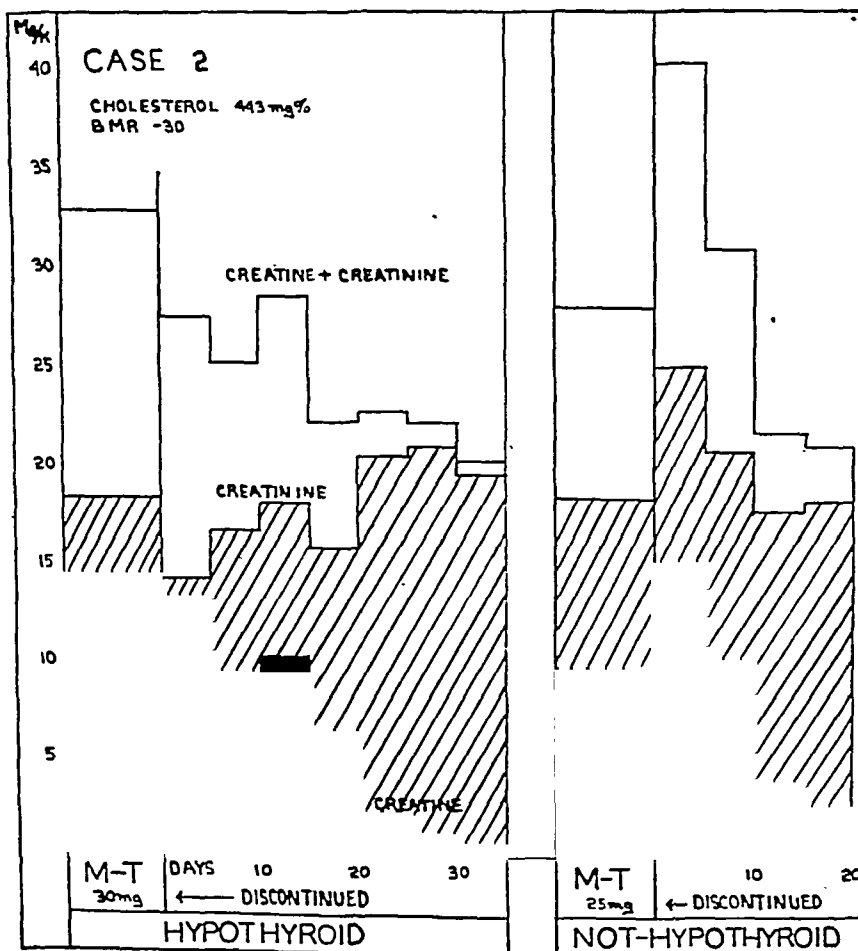
FIG. 7. EFFECTS OF WITHDRAWAL OF METHYL TESTOSTERONE IN HYPOTHYROID PATIENT COMPARED TO EFFECT IN A PATIENT NOT HYPOTHYROID

Each column represents an average of a 5-day period.

When this patient was given methyl testosterone while in a hypothyroid state the excretion of creatine and of creatine + creatinine was high. On discontinuing methyl testosterone, the output of creatine diminished promptly. Subsequently, when adjusted on thyroid medication, the withdrawal of thyroid was followed by a temporary increase in the excretion of both creatine and creatinine such as usually occurs in the normal individual.

by thyroid medication. It is apparent that *thyroid deficiency did not interfere with the synthesis and increased excretion of creatine* which was brought about by methyl testosterone.

In Figure 7 is shown the effect of withdrawing medication with methyl testosterone from patient 2 while she was in the hypothyroid state, in contrast to the results observed when methyl testosterone was discontinued in a hypogonadal patient who was not hypothyroid. *There was no increase* in the output of creatine or creatinine on withdrawing methyl testosterone during hypothyroidism. Instead, the excretion decreased rapidly to a low level. The same observation was made in patient 1.

In Figure 8 we have recorded other observations made on the same hypothyroid subjects. *Patient 1* had been treated with thyroid for nearly three months when the experiment was begun. On a dose of 128 mgm. daily, the creatine output was
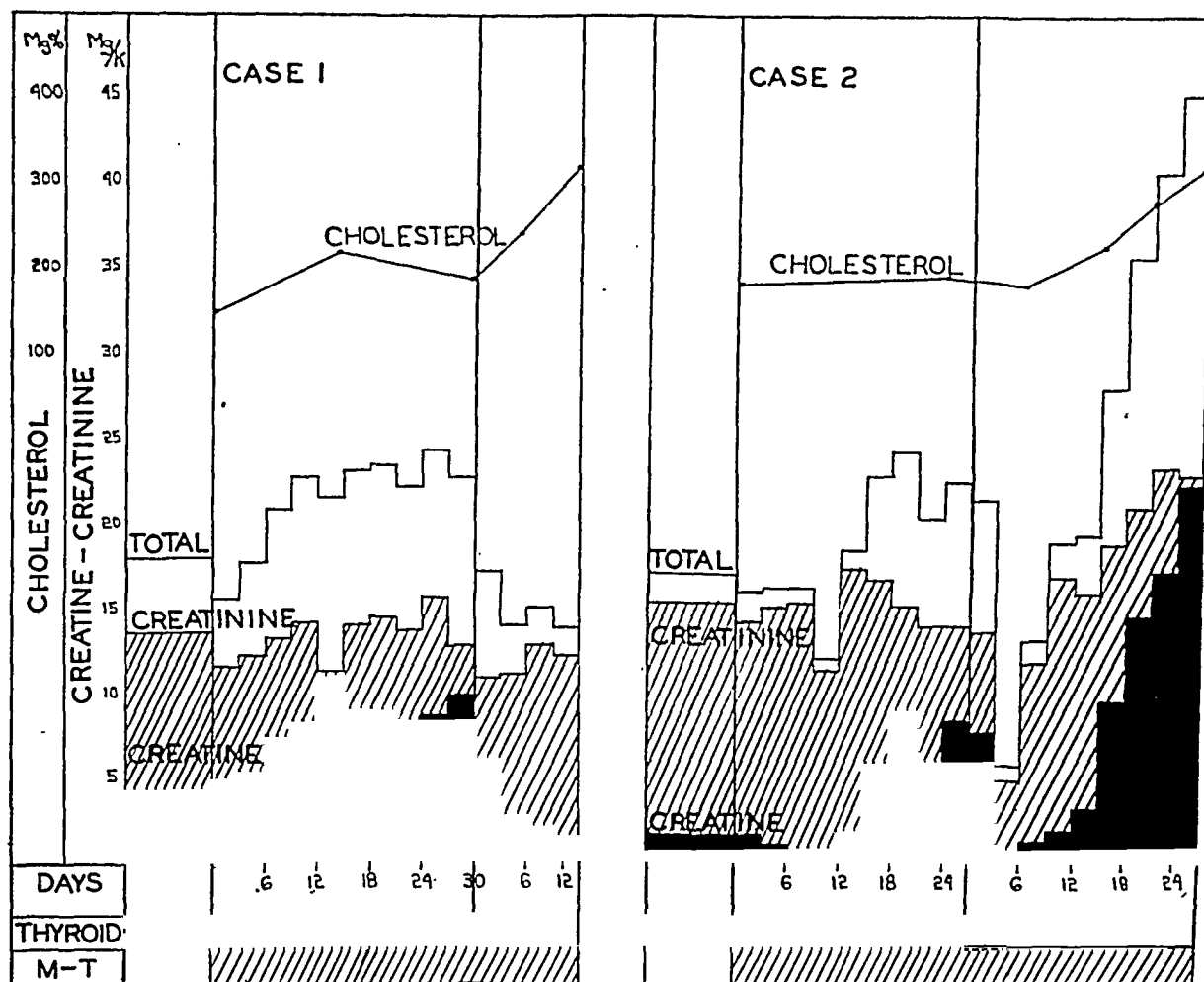
FIG. 8. WITHDRAWAL OF THYROID DURING TREATMENT WITH METHYL TESTOSTERONE

Each column represents an average of a 3-day period.

Case 1. In first period, patient was well adjusted on 128 mgm. thyroid daily and had normal output of creatine and creatinine. The administration of 25 mgm. of methyl testosterone while thyroid was given caused increased output of creatine. After 30 days thyroid was stopped while methyl testosterone was continued. There was an immediate decrease in the output of creatine to a low level during the next 13 days. The serum cholesterol rose.

Case 2. Previous experiment repeated on another patient and continued longer. On the administration of methyl testosterone during thyroid treatment there was a latent period followed by an increasing output of creatine. After 26 days thyroid was discontinued but methyl testosterone continued. Withdrawal of thyroid caused an immediate decrease in the output of creatine for 13 days after which the excretion rose rapidly to higher levels than previously. There was a brief drop in the creatinine during the second 3-day period.

fairly stabilized at 4.5 mgm. per kgm. per day, creatinine 13.7 mgm. per kgm. While he continued to receive the same dose of thyroid, he was given 20 mgm. of methyl testosterone daily for a period of 30 days. The output of creatine rose rapidly and at the end of the period had reached a plateau at 8.5 to 10.0 mgm. per kgm., while the creatinine excretion was 13.0 to 15.8 mgm. per kgm. At this time thyroid medication was discontinued but treatment with methyl testosterone was continued. The output of creatine dropped rapidly by the second day and between the 10th and 13th days was 1.5 mgm. per kgm., while the creatinine was 12.4 mgm. per kgm. Unfortunately, the experiment was discontinued at this point.

Patient No. 2 had previously been stabilized on a daily dose of 96 mgm. of thyroid and had a fairly constant output of creatine, averaging 1.8 mgm. per kgm. with a creatinine excretion of 15.5 mgm. per kgm. Continuing the same dose of thyroid, the patient was given 30 mgm. methyl testosterone daily. During the first 12 days there was a slight decrease in the excretion of creatine and creatinine. After this, the excretion increased until on the 25th and 26th days the output was creatine 8.5 mgm. per kgm. and creatinine 14.4 mgm. per kgm. At this time thyroid medication was discontinued but the administration of methyl testosterone was continued. After 2 days the excretion of creatine decreased abruptly and remained at levels between 0.8 and 2.0 mgm. per kgm. between the 4th and 13th days. After the 13th day the output again increased reaching 9.7 mgm. per kgm. between the 16th and 18th days, and then continued to rise until between the 25th and 27th days the output of creatine was 22.4 mgm. per kgm. and that of creatinine 23.0 mgm. per kgm. It should be noted that in this experiment the creatinuria due to methyl testosterone was on an ascending curve which had not yet reached a plateau. In the midst of this ascent the withdrawal of thyroid caused an abrupt decrease in the output of creatine which lasted for about 13 days. This was followed then by an increasing creatinuria which again followed the ascending curve to the high levels frequently observed under medication with methyl testosterone (compare Figure 3). *The temporary decrease in creatine excretion must be attributed to the onset of thyroid deficiency* brought about by withdrawing therapy, because it has never been observed while methyl testosterone was being administered to individuals who were not hypothyroid. The significance of this finding will be discussed later.

Summary: The various effects of thyroid hormone on the storage and excretion of creatine and creatinine are summarized in Table IV.

DISCUSSION OF CREATINE METABOLISM

In the studies presented above we have dealt entirely with the endogenous metabolism of creatine which has been shown to proceed independently of ingested creatine. The present theories on the relations of creatine in the body are illustrated in Figure 9.

TABLE IV

Summary of effects of thyroid on storage and excretion of creatine bodies

	Muscle		Urine			
	Creatine	Phospho-Cr.	Creatine	Creatinine	Total	Creatine per cent of total
Hyperthyroidism	−	−	+	−	−?	30 to 60+
Hypothyroidism	+	+	−	+	+?	0 to 6 (Normal 10 to 30)

Correction of hyperthyroidism—excretion of creatine and creatinine reversed toward normal
Correction of hypothyroidism
(Urinary Excretion)
    First phase   Creatine ++++ Creatinine − Total +++ (lasting 30 to 60 days)
    Adjusted phase Creatine + Creatinine − Total unchanged or slightly −
Methyl testosterone in hypothyroid patient
  1. Causes increased creatine synthesis and excretion as in normal patient
  2. Withdrawal of M-T causes no temporary increase of excretion
Induction of hypothyroidism (discontinuing thyroid) in patient receiving methyl testosterone
  Causes temporary decrease of creatinuria

Creatine is synthesized by the combination of arginine and glycine to form glycocyamine which is methylated by some agent such as methionine or choline. New creatine is constantly formed to replace the urinary loss of creatine and creatinine. In the muscle, creatine enters into a rapid equilibrium exchange with inorganic phosphate. The enzymatic reactions necessary for this cycle involve the formation of adenosine triphosphate and the oxidation of carbohydrates (6 to 8). The excretion of creatine is apparently influenced by factors different from those which govern the out-

put of creatinine. It may vary markedly in the same individual. It is high in childhood, low in adult life. It is increased by starvation, high protein diet, carbohydrate deprivation, fevers, thyrotoxicosis and by the administration of methyl testosterone, and is decreased in hypothyroidism and by the administration of testosterone propionate. The complete absence of creatine from the urine at times may be due to the fact that there is a renal threshold so that it is excreted only when the serum concentration exceeds 0.58 mgm. per cent (35). In marked contrast the excretion of crea-
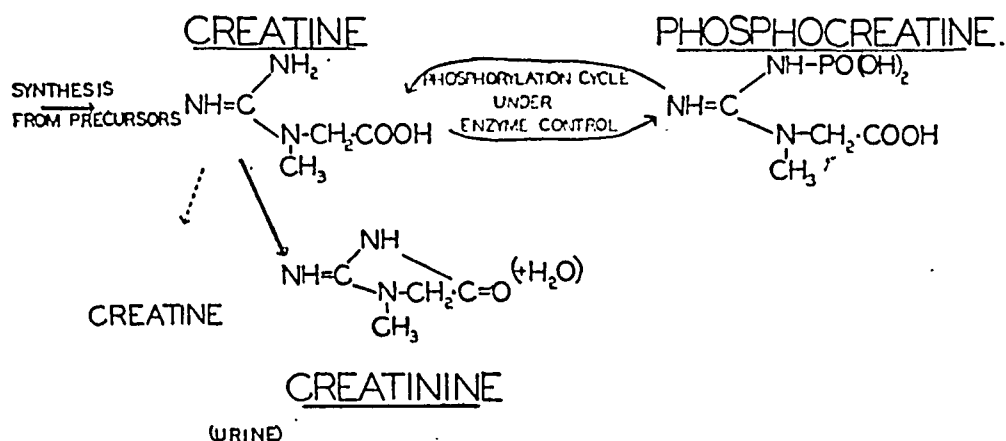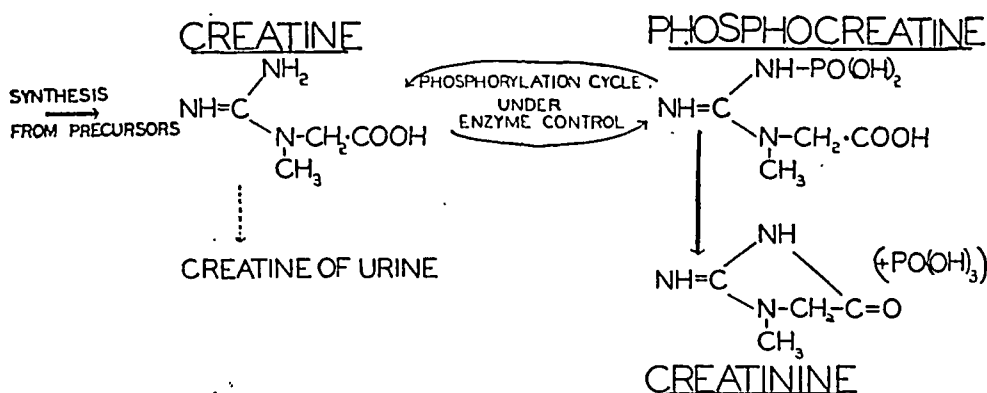
FIG. 9. TWO THEORIES OF CREATINE-CREATININE METABOLISM

Represents the liberation and excretion of creatine. This varies considerably depending upon the ability of the muscles to retain or liberate free creatine. If the amount liberated into the blood is below the renal threshold none appears in the urine.

Represents the formation and excretion of creatinine. The chemical reaction is believed to occur at a constant rate in which case the amount formed would depend upon the total stores of creatine or of phosphocreatine in the body. The excretion is not limited by a renal threshold.

tinine is fairly constant for each individual. The relative stability of the output and the lack of effect of exercise and diet led originally to the concept that the total amount of creatinine excreted depended solely upon the total muscle mass of the body. The fact that changes sometimes occur which are greater than can be accounted for by alterations in the muscle mass makes it seem more probable that the creatinine output depends upon the total stores of creatine in the muscle which might be subject to variation, rather than upon the muscle mass. Bloch, Schoenheimer and Rittenberg (36) showed that the rate of excretion of creatinine corresponded to a conversion of 2 per cent of the creatine in the body in 24 hours. There is no renal threshold for creatinine.

It has been generally accepted that creatinine is derived from creatine by the removal of a molecule of water and the addition of a C-N linkage (Theory A. Figure 9). Recently a number of workers have suggested that creatinine might be formed by the dephosphorylation of phosphocreatine as shown in Theory B. Figure 9. Borsook and Dubnoff (37) found that *in vitro* at 38° C. without the intervention of an enzyme, phosphocreatine breaks down to creatinine at the rate of 2 per cent in 24 hours, which is much faster than the formation of creatinine from creatine under the same conditions. Rosengart (38) working with minced muscle obtained similar results and Lipmann (8) suggested the same possibility. Wang (4) as a result of extensive clinical investigations, stated "the formation of creatinine may be somehow related to the amount of phosphocreatine present in the organism and not to the total creatine contents."

Taking the above facts into consideration, it is apparent that changes in the excretion of creatine or of creatinine might result from a number of different causes, namely:

1. *Changes in the rate of synthesis of creatine from its precursors*

It has been shown that the synthesis of creatine is increased by the administration of methyl testosterone (13, 14). Other conditions which might affect this synthesis have not been fully investigated.

2. *Changes in the ability of the muscles to store or liberate creatine or phosphocreatine*

Analyses of muscles have shown that the concentration of creatine is decreased in thyrotoxicosis (4, 26, 27), in progressive muscular dystrophy (39, 44) and in denervation atrophy (41). Wang (4) demonstrated increased creatine and phosphocreatine in the muscle of thyroidectomized rabbits, and Williamson and Gulick (42) found increased total creatine of muscle after the administration of testosterone propionate.

3. *Changes in the rate or direction of the creatine-phosphocreatine cycle*

Direct studies on the ways in which this reaction may be influenced are lacking. However, several workers (43, 4) have suggested that thyroid may have an influence on the synthesis or breakdown of phosphocreatine.

4. *Changes in the rate of conversion of creatine or of phosphocreatine into creatinine*

We are not aware of any direct evidence on this subject.

CONSIDERATION OF THE EFFECTS OF THYROID ON CREATINE METABOLISM

Does thyroid influence the rate of synthesis of creatine from its precursors?

The observations which we have recorded suggest that it has little, if any, influence. In the two hypothyroid patients studied (see Figure 6) methyl testosterone caused increased excretion of creatine irrespective of whether the condition was untreated or well controlled by thyroid therapy. Furthermore, treatment of hypothyroid patients with methyl testosterone caused an increased output of creatine + creatinine, whereas after the first phase of adjustment thyroid medication did not increase but possibly diminished the total output, although the ratio of the two compounds was altered (Figure 2).

Does thyroid influence the storage of creatine?

We have already discussed the evidence that the large amounts of creatine excreted during the first 30 to 35 days of thyroid medication by the four patients presented in Figure 2 were apparently due to the release of stores of muscle creatine. In normal individuals thyroid causes a simi-

lar response, but larger doses are required and the losses of creatine are smaller and of shorter duration (31). We have reported (44) that thyrotropic hormone acts in the same way in most normal individuals.

When thyroid is withdrawn from the body increased amounts of creatine are apparently retained. This is suggested by the experiment (Figure 8) in which treated myxedematous patients were allowed to lapse into the hypothyroid state by withdrawing thyroid therapy while they continued to receive methyl testosterone. Even though the rates of synthesis and excretion of creatine were increased greatly above the normal by methyl testosterone, the withdrawal of thyroid apparently caused creatine to be retained to such a degree that little was excreted until after the 13th day, when the storage capacity of the muscles again became filled to overflowing. Additional evidence that the hypothyroid patient retains creatine more tenaciously than the normal individual is afforded by the observation that when methyl testosterone was discontinued there was no release of a portion of the stored creatine such as occurs in the normal individual (Figure 7). These deductions concerning the effects of thyroid on the storage of creatine are substantiated by the careful chemical analyses of the muscles of rabbits by Wang (4) showing that in hypothyroidism the concentrations of creatine and phosphocreatine are increased and in hyperthyroidism they are diminished.

Little is known concerning the way in which thyroid governs the movements of creatine into or out of the muscles. It is possible that the loss of creatine which occurs when the hypothyroid patient is first treated may to some extent be accounted for by the breakdown of muscle tissue under the influence of thyroid. We have corroborated the observation of Maroney and Johnston (45) that the administration of thyroid to hypothyroid children results at first in a negative nitrogen balance which is then followed by a positive balance as the patient begins to grow and build new tissue. As shown in Figure 2 the periods of nitrogen deficit correspond roughly to those of creatine loss. However, the amount of muscle protoplasm catabolized calculated from our data [2]

on the assumption that all the nitrogen loss comes from muscle tissue, is insufficient in some cases to yield the amounts of creatine + creatinine which were lost in these periods. Likewise it is difficult to believe that the increased stores of creatine which are accumulated when thyroid is withdrawn can be explained entirely by the building of new muscle protoplasm. Another possibility is that the movements of creatine may be associated to some extent with those of tissue fluid. This is suggested by the fact that the changes in creatine excretion and the loss or accumulation of myxedematous fluid are the earliest and most rapid effects observed when thyroid is given or withdrawn. They occur much more rapidly than changes in the B.M.R. More extensive study would be necessary to determine whether the movement of creatine into or out of muscle occurs independently or whether it is associated with changes in muscle protoplasm or tissue fluid.

Does thyroid influence the rate of conversion of creatine into creatinine?

Both our data and the studies of others offer convincing evidence that the amount of creatine which is converted into creatinine is decreased in hyperthyroidism and increased in hypothyroidism. It is not possible to explain the diminished output of creatinine caused by thyroid as the result of a decrease in the total muscle mass, because when a hypothyroid child is properly treated he stores nitrogen, grows and develops increased musculature while his creatinine output decreases. Likewise if the change in creatinine output were due to a catabolic effect of thyroid on the muscle tissue, one would expect the urinary creatinine to be high in hyperthyroidism, whereas actually it is low.

If the amount of creatinine formed daily depends solely upon the total stores of creatine or of phosphocreatine in the body, the changes in excretion observed might depend upon alterations in stores which are brought about by thyroid. Thyroid increases the output of creatine, thereby diminishing the stores in the muscles and hence the production of creatinine. Deficiency of thy-

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[2] Because of the variability of the fluid content of myxedematous muscles, only an approximate calculation can be made from the nitrogen losses of the amounts of creatine which might be liberated by the breakdown of muscle protoplasm. It is possible that in cases 2 and 4, sufficient muscle was catabolized to yield the amounts of creatine lost, but this is not true in cases 1 and 3.

roid leads to increased stores in the muscle and accordingly increased production of creatinine. These alterations in the excretion of creatinine could occur irrespective of whether it is derived from creatine or phosphocreatine, because the stores of both creatine and phosphocreatine are increased or decreased simultaneously.

It is by no means certain that the changes in the muscle stores are of sufficient magnitude to account for the degree of alteration in the output of creatinine actually observed. In this case, it is necessary to assume that thyroid must in some way influence directly the conversion of creatine into creatinine. If creatinine is derived directly from creatine, this reaction must be directly affected. On the other hand, if creatinine is formed from phosphocreatine, thyroid might diminish the production of creatinine by influencing the enzyme system controlling the phosphorylation of creatine to phosphocreatine as suggested by Wang.

Our observations offer no solution to these problems but indicate the necessity of further investigations.

SUMMARY AND CONCLUSIONS

We have discussed the present knowledge of the metabolism of creatine especially in reference to the problems of storage and excretion, and have presented two theories concerning the origin of creatinine (Figure 9). It seems probable that alterations in the excretion of creatine bodies might be brought about by changes (1) in the rate of synthesis of creatine from its precursors; (2) in the ability of the muscles to store or to liberate creatine; (3) in the rate or direction of the creatine-phosphocreatine cycle; (4) in the rate of conversion of creatine or of phosphocreatine into creatinine.

Various studies of the effects of thyroid hormone on the storage and excretion of creatine and creatinine are reported and summarized in Table IV. Analysis of these observations suggests the following conclusions:

Thyroid has negligible effect upon the synthesis of creatine from its precursors. Its effect upon the excretion of creatine depends largely upon the fact that it facilitates the loss of creatine from the muscles. Excess of thyroid increases the amount of creatine liberated from the muscles,

thereby decreasing the stores of creatine and phosphocreatine. In the absence of thyroid, creatine is retained in the muscle and the stores of both creatine and phosphocreatine are increased. When thyroid is administered to a patient with myxedema the amounts of creatine lost cannot be accounted for entirely by the catabolism of muscle protoplasm. It is not known whether the movements of creatine into or out of muscle occur independently or are associated with changes in muscle protoplasm or of tissue fluid.

The fact that the output of creatinine is altered in the opposite direction from that of creatine might be explained solely by the changes of concentration of creatine and phosphocreatine in the muscles. However, it is not certain that the magnitude of the changes in concentration of the muscle stores is sufficient to account for the change in the excretion of creatinine actually observed. It is possible that in addition to diminishing the stores of creatine and phosphocreatine in the muscles, thyroid may also decrease the conversion of these substances into creatinine.

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A SURVEY OF THE TWENTY–FOUR–HOUR URIC ACID AND UREA CLEARANCES IN ECLAMPSIA AND SEVERE PREECLAMPSIA [1]

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The uric acid clearance in eclampsia (1) and in severe preeclampsia (2), has recently been shown to be decreased. Similarly, earlier work had shown the urea clearance in this disease to be decreased (3 to 6). More recently, kidney function tests (clearance) performed with such foreign substances as inulin, diodrast and phenol red (PSP) have uniformly shown a decreased glomerular filtration rate as the outstanding alteration in kidney function found in the late toxemias of pregnancy by these techniques (7 to 9). One might expect, therefore, a general increase in the level of several blood constituents in the late toxemias of pregnancy as a consequence of this decreased glomerular filtration rate. Yet there are patients who seem to have normal N.P.N. and urea values with definite elevations in the uric acid levels.

We decided, therefore, to study further the uric acid and urea clearance in the late toxemias of pregnancy in order to determine if there were some cases which are classified clinically as either eclampsia or severe pre-eclampsia which might have a normal kidney function with respect to one metabolite, such as urea, and an abnormal or decreased function with respect to another metabolite, such as uric acid. We wished, further, to obtain as continuous a picture of the kidney function as is possible so that the changing blood levels of both the uric acid and the urea might be correlated with the clearances obtained.

The short clearance periods usually employed will yield exact information as to the kidney function during the time (at best 1 to 2 hours) the test is performed. Such data might justifiably be considered as a close approximation of the kidney function for some hours preceding and following the test if it is assumed that the rate of change in this parameter is rather slow. Now it is obvious clinically that in the toxemias of pregnancy kidney function may change markedly from day to day and in some instances from hour to hour. Under such circumstances the usual clearance technique will yield information which is representative of the kidney function only at one particular moment.

Furthermore, if one is interested in obtaining a continuous picture of kidney function during the acute phase of the disease and during the recovery period, one might conceivably perform short period clearances daily during both the active phase of the disease and the puerperium. However, in considering a survey study such a procedure seemed both impracticable and, from the point of view of the welfare of the patient, definitely contraindicated.

It was decided, therefore, to collect 24-hour urines and to calculate the clearances on this basis. The accuracy of such clearances as compared with the short period clearances is probably reduced and these clearances may yield only figures which are of the correct order of magnitude and not the exact values. In spite of the potential sources of error which will be discussed below, the 24-hour clearances do yield information which may be obtained practically, as to the average 24-hour performance of the kidney and as to the relative rate of change in this function.

This report deals with the uric acid and urea clearances obtained by this procedure during the years 1942, 1943, and 1944, on 32 cases of late toxemia of pregnancy (eclampsia and severe pre-eclampsia).

SUBJECTS AND METHODS

The data reported herein were obtained upon 3 cases of antepartum eclampsia, 2 cases of questionable postpartum eclampsia and 27 cases of severe pre-eclampsia. The pertinent obstetrical data relating to these cases are given in Table I. Only the uric acid clearances were determined on the first 7 cases. Both the uric acid and urea clearances were obtained on the remaining 25. The regimen upon which these patients were placed prob-

TABLE I

Obstetrical histories of patients studied

Case no.	Initials	History no. N.Y.H.	Age	Week of pregnancy	Parity	Complication
1	M.P.	312860	27	34	1-1-1	SPE
2	E.D.	308678	25	38	1-1-1	SPE
3	C.C.	319565	29	39	1-1-1	SPE
4	M.C.	328757	44	40	7-7-6	SPE
5	M.B.	317370	20	29	1-1-1	SPE
6	C.R.	318210	29	32	1-1-1	E
7	H.R.	323208	21	32	1-1-1	E
8	C.S.	331232	28	27	0-1-0	SPE
9	J.U.	164200	24	34	2-2-1	SPE
10	B.S.	297718	23	36	1-2-1	SPE
11	M.H.	334290	27	40	1-1-1	SPE
12	H.P.	340692	27	37	1-1-1	SPE
13	J.P.	343526	26	33	2-2-2	SPE
14	R.O.	345159	31	33	1-1-1	SPE
15	L.E.	345953	24	37	1-1-1	E
16	M.C.	272867	37	36	2-3-3	SPE
17	V.K.	347785	33	39	2-4-2	SPE
18	J.N.	333272	28	30	0-2-0	SPE
19	M.S.	366390	35	34	2-2-1	SPE
20	V.V.	360393	33	35	2-2-2	SPE
21	J.R.	376076	28	31	1-1-0	SPE
22	J.O.	369460	27	29	0-1-0	SPE
23	A.V.	348308	35	36	9-11-6	SPE
24	S.M.	367203	33	37	2-2-2	SPE
25	G.D.	373202	36	38	3-4-3	SPE
26	B.B.	102727	41	38	4-4-4	SPE
27	A.P.	384802	18	29	0-1-0	SPE
28	A.M.	383855	25	35	1-3-1	SPE*
29	M.L.	380679	39	38	1-1-1	SPE
30	H.M.	375749	27	40	1-1-1	Tox.-Unc.
31	M.S.	382530	21	38	1-1-1	PPE
32	M.M.	279291	27	32	0-4-0	SPE

E = Eclampsia; SPE = Severe preeclampsia; PPE = Postpartum eclampsia; Tox.-Unc. = Toxemia, unclassified.
* Also had PPE.

The clearances were calculated in the usual way from the following data. The urine concentration of the substance in question was obtained by an analysis of a suitable aliquot of the 24-hour urine. The average plasma concentration was approximated by averaging the value obtained in blood drawn at the beginning and at the end of the 24-hour period. In the less acutely ill patients these blood specimens were taken before breakfast; those acutely ill might have been receiving an infusion of 20 per cent glucose in distilled water shortly before the blood was drawn. The urine flow was calculated by dividing the corrected urine volume in ml. by 1440 minutes. At times, particularly during the post partum period when it was not necessary to obtain a blood specimen daily, the value for the days on which the blood was not taken was determined by interpolation and subsequent averaging as described above. All clearances are corrected to 1.73 sq. m. surface area.

RESULTS

The 24-hour uric acid clearances obtained in this way during the active phase of the disease averaged 6.4 ml. per minute, and during the postpartum or early puerperium period, 10.4 ml. per minute.[2] The difference between these two average values and the differences between these and the average normal 24-hour uric acid clearance are statistically significant.

Similarly, the 24-hour urea clearance during the active phase of the disease averaged 42 ml. per minute, and during the recovery phase, 55 ml. per minute.[2] Again the difference between these two average values and the differences between these and the average normal 24-hour urea clearance are statistically significant.

The average postpartum clearances in the toxemia group should not be expected to reach normal values when all the postpartum data are averaged, since the clearances do not reach normal values until the third to fourth postpartum day.

The normal 24-hour uric acid clearance is considered to be about 12 ml. per minute. This figure has been arrived at by averaging 39 separate 24-hour periods obtained at various times upon 7 different patients who were being fed the usual hospital diet. There seems to be no appreciable difference between pregnant and non-pregnant women.

ably does not modify the clearances except as discussed below.

The 24-hour urines were collected in the usual manner. However, it is not always possible to obtain complete 24-hour specimens under these conditions. Therefore, the creatinine content of all the urines was determined and the urine volumes were corrected subsequently to an approximately constant creatinine excretion which seemed to be characteristic for the particular individual.

Blood specimens were obtained by venipuncture, generally of the cubital vein.

Uric acid was determined in Wu filtrates of plasma and in suitably diluted specimens of urine by the 1933 Folin method (10) in the first part of the work, and later by the 1922 Folin method (11) modified as previously described (12). If it was necessary to deproteinize the urine, the deproteinization was performed as described by Schaffer, et al. (2).

Urea was determined by the hypobromite manometric method of Van Slyke and Kugel (13).

Creatinine was first determined by the Folin method (14) and subsequently by the method described elsewhere (15).

[2] Clearances within normal limits during the active phase of the disease or definitely subnormal after the 4th postpartum day as described below are not included in these averages.

| Clearance | Toxemias | | Normal |
	Antepartum	Postpartum	
24 hr. UAC	6.4±2.0	10.4±2.1	12.4±2.4
$\frac{m_1-m_2*}{\sigma_D}$		15.8	4.8
24 hr. UC	41.8±13.7	54.7±13.0	63.4±8.7
$\frac{m_1-m_2}{\sigma_D}$		33.1	13.0
Short UAC		14.5±2.7	14.0±2.9
$\frac{m_1-m_2}{\sigma_D}$			V.S.
Short UC		75±13	76±13
$\frac{m_1-m_2}{\sigma_D}$			V.S.

UAC—Uric acid clearance.   UC—Urea clearance.

* $\frac{m_1-m_2}{\sigma_D}$—a measure of the significance of the difference between the two values to the right and left immediately above. A numerical value greater than three is considered highly significant.

V.S. = very small, i.e., not significant.

The normal 24-hour urea clearance (29 periods on 6 different patients) averages 63 ml. per minute, with all clearances calculated as maximal clearances. (Complete numerical data are presented in Table II).

To determine the relationship between the clearance and the blood value of uric acid and urea, the coefficients of correlation between these values have been calculated. None of the data has been excluded. As might be expected, a significant negative correlation is found between the uric acid clearance and the plasma uric acid. (Numerical data are presented in Table III.) Similarly, a significant negative correlation is found between the urea clearance and the blood urea nitrogen. Presumably, if the few cases cited below which are exceptions to the general rule were omitted from the calculations, even better correlations would be obtained. Further, there is a significant positive correlation between the urea clearance and the uric acid clearance. It seems, therefore, that these overall data may be taken to indicate a decreased ability on the part of the kidney to excrete urea and uric acid. They also indicate a definite relationship between this kidney function and the blood levels of both uric acid and urea.

However, when the cases are considered individually certain exceptions to this general behavior are found. The majority of the patients on which there are sufficient data (17 out of 25), have both low uric acid and urea clearances during the active phase of the disease. Both these clearances have become essentially normal by the third to fourth postpartum day. In some cases there is a slight decrease in the uric acid clearance following this initial rise, with a subsequent gradual rise in the clearance to essentially normal values. Cases No. 12, 13, and 27 in Table IV are presented as examples.

There were 4 cases (No. 9, 20, 21 and 22) in which the uric acid clearance remained low during the course of the disease and returned to normal during the early puerperium, as those above. But the urea clearance seemed to remain within normal limits at all times. Case No. 9 is presented

TABLE III

Numerical data from statistical evaluations of 24-hour clearances

x	y	Mx	My	N	σx	σy	r	t	Equation
UAC	PUA	8.16	6.61	408	3.29	1.57	−0.62	16.02	y = 9.03 − 0.03 x
UC	BUN	51.9	11.46	250	17.28	3.41	−0.56	10.63	y = 17.17 − 0.11 x
UC	UAC	51.9	7.9	250	17.28	3.21	+0.47	8.41	y = 3.42 + 0.87 x
V	UAC	1.23	8.16	408	0.65	3.53	+0.16	2.44	y = 7.09 + 0.87 x
V	UC	1.27	51.9	250	0.65	17.28	+0.28	4.60	y = 42.40 + 7.44 x
V	UC	1.11	50.4	222	0.42	27.27	+0.16	2.40	y = 38.91 + 10.38 x

UAC = Uric acid clearance   PUA = Plasma uric acid   UC = Urea clearance
BUN = Blood urea nitrogen   V = Urine flow   N = Number of clearances
Mx = Mean of x   My = Mean of y   σx = Standard deviation of x
σy = Standard deviation of y   r = coefficient of correlation   t = "t" value. An index of significance,
calculated as follows $t = \frac{r}{\sqrt{1-r^2}} \cdot \sqrt{N-2}$.

TABLE IV

Examples of uric acid clearance, plasma uric acid, urea clearance, and blood urea nitrogen in patients with severe preeclampsia

Patient		Days antepartum												DD	Days postpartum													
		12	11	10	9	8	7	6	5	4	3	2	1	DD	1	2	3	4	5	6	7	8	9	10	11	12	13	14
M.P. #12	UAC											7.1	5.0	3.7	3.6	6.6		10.4	12.0	12.5	11.3	10.0						
	PUA											6.7	6.8	7.0	7.4	8.0		7.2	5.8	5.0	4.8	4.0						
	BUN											10.7	7.7	8.0	8.2	10.4		6.7	6.7	7.2		8.5						
	UC											47	38	29	31	40		76	76	70		64						
J.P. #13	UAC										6.4	4.7	4.0	4.0	7.2	11.4	13.7		10.7									
	PUA										7.1	7.4	7.5	7.7	9.0	7.0	6.2		5.0									
	BUN										8.3		10.4	14.2			9.1		8.1									
	UC										38		42	31			79		75									
A.P. #27	UAC										2.1	2.3	2.2	3.1	6.9	10.7			9.4	8.3		12.0	12.6	9.1		9.3	10.0	
	PUA										9.6	9.9	10.8	11.9	11.0	8.8			6.2	6.0		5.3	5.2	5.1		4.9	4.8	
	BUN										18.5	20.3	16.5	14.0	12.3	11.0			10.1	9.9		12.2	11.9	11.3			12.5	
	UC										10	15	28	31	43	48			63	43		46	55	48			43	
J.U. #9	UAC				7.3	7.9	6.9	7.3	5.8	5.2	8.6	8.4	6.5	6.3	8.9	8.8	9.0	9.6		11.8	13.5							
	PUA				8.0	8.1	7.3	7.9	5.2	6.0	6.2	6.0	6.2	6.4	6.5	6.6	6.8	6.4		5.8	5.0							
	BUN					9.3	8.0	7.9	6.5	6.0			6.6	6.1	5.0	6.3				9.4	10.5							
	UC					57	65	64	67	62			56	59	74	62				63	65							
R.B. #26	UAC													17.4	17.9		13.8	10.8	13.4	18.5	13.2	13.3						
	PUA													3.7	3.7		5.8	5.1	5.1	4.5	4.1	4.2						
	BUN													9.2	9.7		13.2	13.2	14.6	16.6	17.9	18.7						
	UC													45	75		45	41	55	46	42	42						
A.V. #23	UAC			8.5	7.9*	7.9	8.5	8.7	6.8	6.7	7.4	6.8	5.7	5.0	4.7	4.8	4.3	5.1		4.9	6.2	5.9		6.4	5.7			
	PUA			8.4	8.0	7.8	7.9	7.4	8.5	8.4	8.4	8.2	8.0	8.8	9.9	9.9	9.5	9.1		8.2	8.0	8.0		8.0	8.0			
	BUN			6.3	8.5	7.7	9.4	9.1	7.9	9.0	8.9		10.0	11.2	12.4	13.3	14.1	12.8		13.0	14.4	15.9		15.8	15.0			
	UC			62	60	54	63	56	63	56	84		51	50	41	49	44	62		45	56	55		57	47			
J.O. #22	UAC	7.4									6.9	8.2	10.3	10.0	11.4			11.9			10.2	9.4	10.3	10.4		11.8		
	PUA	7.2										6.5	6.2	5.8	5.4			6.0			6.1		5.7	5.7		6.1		
	BUN	8.1										10.1	9.9	10.1				9.8										
	UC	67										58	88	82				79				86						

UAC = Uric Acid Clearance, ml. per min.; PUA = Plasma Uric Acid, mgm. per cent.; BUN = Blood Urea Nitrogen, mgm. per cent; UC = Urea Clearance, ml. per min.
* Fetal Heart Lost.

as an example in Table IV. It will be noted that in this case (and in the others) the blood urea nitrogen remained well within normal limits for a pregnant woman at term.

There were two cases (No. 26 and 31) in which the uric acid clearance remained within normal limits at all times, but the urea clearance decreased during the puerperium. In both these cases the blood urea nitrogen was increasing while the plasma uric acid remained essentially constant or decreased somewhat. Case No. 26 is presented as an example in Table IV.

And finally there were 2 cases (No. 23 and 28) in which the uric acid clearance was low during the active phase of the disease and remained low during the puerperium. In one of these the urea clearance was nearly normal but in the other it was definitely low. This last patient exhibited clinical signs and symptoms of "late postpartum eclampsia" and will be described in detail in another publication. Case No. 23 is presented as an example in Table IV.

If the fetal heart is lost these changes take place exactly as if the uterus has been emptied. The subsequent delivery of the dead fetus does not alter the clearances which remain normal. Case No. 22 is presented as an example in Table IV. In this and another instance (Case No. 21) the urea clearance remained within normal limits, but in two other cases which were not followed daily until the dead fetus had been delivered (Cases No. 8 and 22) both the uric acid and urea clearances were low and improved following the loss of the fetal heart as in the majority of the other cases cited above.

Ordinary short period uric acid and urea clearances were obtained on the majority of these cases during the early puerperium (5 to 15 days postpartum). These data are presented in Table V. In every instance they confirm the data obtained by the 24-hour clearances. It will be seen that the uric acid and urea clearances are within normal limits in most of the cases. The average of the uric acid clearances (excluding the two low values in parentheses) is 14.5 ml. per minute. This value is practically identical with the value previously reported by us for clinically normal women during the early puerperium (12).

The urea clearances (again excluding those values in parentheses) average 75 ml. per minute.

TABLE V

Short period uric acid and urea clearances—postpartum

Case no.	Days postpartum	UAC	UC
		ml. per min.	*ml. per min.*
7	8	10.1	56
8	8*	13.3	74
9	7	16.2	75
12	12	16.0	85
13	7	16.1	82
14	8		(113)
15	7	11.7	88
16	9	16.8	85
17	5		84
18	6		74
19	5		66
20	6	13.4	(113)
21	6	14.5	78
22	8	10.4	86
23	7	(6.6)	66
24	12	13.6	(113)
25	9	19.2	99
26	9	13.5	(44)
27	15	10.0	63
28	6**	(7.6)	57
29	6	14.3	53
31	15	18.7	82

* Days following the loss of the fetal heart
** One day before convulsions.

Again this is about the same value as that obtained on clinically normal women (12).

It will be noted that the aberrant low uric acid clearances were obtained upon the patients who also had low 24-hour clearances and the low urea clearances similarly upon that one patient who exhibited low 24-hour clearances.

The three high urea clearances (113 ml. per min.) cannot be explained.

The only really low urea clearance was obtained on one of the two cases which exhibited a decreasing clearance during the early puerperium with a normal uric acid clearance. Since the other similar case had normal clearances for both uric acid and urea fifteen days postpartum, one could suppose that the same would occur in this case, too, if another clearance had been obtained.

It appears, therefore, that in general the uric acid and urea clearances are low during the active phase of the disease, and that both these clearances return to normal in the early puerperium. Since there is a good correlation between these clearances and the blood levels of uric acid and urea, it is probable that a large portion of the increases in blood level of both uric acid and urea in severe preeclampsia and in eclampsia is due to a decreased kidney excretory function. Certain cases

do appear, however, which are classified clinically as severe preeclampsia, which are distinct exceptions to this general behavior.

DISCUSSION

Before any attempt is made to interpret and evaluate the above data, a defense of some of the more obvious criticisms of this type of procedure should be made. Since a day by day picture of the kidney function with respect to uric acid and urea during both the active and the recovery phase of the disease was wanted, the procedure which seemed to be the best compromise was chosen— the use of 24-hour periods. But it has already been pointed out that it is nearly impossible to obtain complete collections of 24-hour urines during the active phase of the disease. And it is just as impractical to conduct the regular short period clearances each day upon such patients. We were forced to assume, therefore, that the creatinine content of the urine could be used as a guide to the accuracy of the collection of the 24-hour urine.

There is evidence in the literature which justifies our making this assumption. Smith (16) has shown that the creatinine excretion is essentially normal in severe preeclampsia. Our own experiments tend to confirm these observations for we have had some few cases in which the collections were adequate as judged by the constancy and the total amount of creatinine excreted. Even if the amount of creatinine excreted daily in severe preeclampsia is constant and normal, it does not justify our correcting incomplete specimens to the correct 24-hour volume unless it is known that the rate of excretion of creatinine is constant over the whole 24-hour period. Though it is so in the normal, it may not be so in these cases, but the variations are probably not sufficiently large to introduce large errors. A more serious error is probably introduced into the uric acid clearance by this correction since it is known that there is a diurnal variation in the excretion of uric acid.

On the other hand, severe preeclampsia is not an all-or-none phenomenon, since clinically there are actually degrees of severity of severe preeclampsia. So it is possible that the creatinine excretion is not constant in all cases. Some of our recent data show this to be definitely true. For example, the creatinine excretion during short clearance periods on a patient with "fulminating"

severe preeclampsia was approximately 80 to 90 per cent of normal, with a urea clearance of 40 per cent of normal.

In general, however, any error due to correcting the urine volume to constant creatinine excretion tends to reduce the differnce between the clearance value obtained during the active phase of the disease and that observed during the recovery phase, or that observed in normal women.

Secondly, many of these patients have received, at various times, infusions of 20 per cent glucose in distilled water. We have observed recently, in qualitative agreement with Talbot (17) and contrary to our previous observations made following a single injection of 50 ml. of 50 per cent glucose, that the continuous infusion of hypertonic glucose may increase the excretion of uric acid. These observations are published in detail in the following paper (18).

However, any increase in the uric acid clearance due to the presence of glucose will again tend to minimize the difference between the antepartum and the postpartum or normal uric acid clearances.

Thirdly, the fluid intake of these patients is limited. The urine flows can therefore be expected to be low. Our overall data indicate, however, that the low clearances are not due to the low flows only, since low clearances have been observed in many cases in spite of relatively high urine flows. Moreover, the coefficient of correlation between the urine flow and the uric acid clearance (all the data) and the urine flow and the urea clearance (both all the data and for flows of 2.00 ml. and less) are low and of the same order of magnitude. It would appear, therefore, that the effect of the urine flow upon the clearances would be of about the same order of magnitude in both instances (for numerical data see Table III).

And lastly, it is not known how far the diet in these cases affects the uric acid clearance. We do know that the cases of mild preeclampsia which are receiving the same diet as those cases of severe preeclampsia do not exhibit these marked changes in uric acid clearance.

In spite of these potential sources of error the data have been of definite value. They show that wide variations in the clearances of these substances may occur. And in spite of the potential sources of error the agreement which has been obtained between the 24-hour clearances and the

short period clearances when these have both been performed on the same day, have in general been much better than has been expected, during both the active phase of the disease and the puerperium.

The data presented, even if not composed of exact values, are suggestive of the actual changes which occur in the kidney function. One is forced, therefore to the tentative conclusion that the hyperurecemia of eclampsia is due in part to a decreased excretion of uric acid by the kidney. To conclude that these changes in clearance prove the hyperuricemia of eclampsia to be only due to kidney dysfunction requires the assumption be made that the rate at which uric acid is produced (or destroyed or utilized) remains constant. There are no data at hand at the present time to justify such an assumption. Therefore, the inverse relationship which is seen to exist between the uric acid clearance and the plasma uric acid level may be taken only as presumptive evidence which indicates that a decrease in kidney function is one of the factors operating toward the maintenance of a hyperuricemia. However, the significant positive correlation between the uric acid clearance and the urea clearance, and the significant negative correlation between the urea clearance and the blood urea nitrogen, tend to offer additional evidence to support the contention that a part of the elevation seen in both the blood uric acid and in the blood urea is due to a decrease in kidney function.

In terms of the present day concept of kidney function, this decrease in uric acid and urea clearance which is observed in the majority of cases can probably be ascribed to a decrease in the glomerular filtration rate. The maintenance of a normal rate of creatinine excretion can be attributed to an increased tubular secretory activity. Such an interpretation is in complete accord with the observations made previously by other methods in this and in other laboratories (7 to 9).

Whether there is or is not a decrease in the glomerular filtration rate in those cases in which there is a decreased uric acid clearance and a normal urea clearance, or *vice versa,* cannot be determined from these data. Regardless of this particular point, one must postulate that wide fluctuations in the tubular reabsorption rate occur in these instances if one is to ascribe the observed phenomena to alterations in kidney function. The

answer to this question can be had by determining the glomerular filtration in patients who exhibit these contrasts. We are now collecting such data. A few preliminary experiments already performed indicate that deviations from normal tubular activity do occur in certain cases of severe preeclampsia.

SUMMARY AND CONCLUSIONS

The 24-hour uric acid clearance has been determined daily in 32 obstetrical patients with either eclampsia or severe preeclampsia as a complication during the course of the disease and during recovery from the disease. The 24-hour urea clearance has also been determined in 23 of these cases. The uric acid and urea clearances were found to be subnormal during the active phase of the disease and to return to nearly normal values by the third to fourth postpartum day in the majority of cases (15 out of 23). However, certain exceptions to this general behavior were found. Four patients showed subnormal uric acid clearances during the disease, which improved during the early puerperium as above. But the urea clearances remained normal throughout the period that these patients were under observation. Two patients showed apparently normal uric acid clearances and urea clearances during the antepartum, but decreasing urea with normal uric acid clearances during the early puerperium. And finally, two others had subnormal uric acid clearances during both the antepartum and the early puerperium. In one of these cases the urea clearance was a low normal while in another it was definitely subnormal. Short period uric acid and urea clearances had become normal in the majority of cases in the early puerperium. Those cases in which the short period clearances were not normal were the same as those few which exhibited abnormal 24-hour uric acid clearances. The sources of error and the possible interpretations of these data are discussed.

It is concluded that there may be a hyperuricemia or a hyperuremia, or both, in eclampsia and severe preeclampsia, and that these elevations may be due largely to an altered kidney function. A decrease in the glomerular filtration rate may serve to explain the phenomena in the majority of cases. However, some of the exceptional cases can be explained only by postulating a change in

the rate of tubular reabsorption of uric acid and urea. The possibility also exists that some of these changes may be due to alterations in the metabolism of these substances.

We wish to express our thanks to the many members of the house and nursing staffs whose cooperation has made this study possible. Many of the analyses were performed by Miss Hertha H. Taussky, M.S., some of the urea analyses by Mr. Nelson Osterberg, and the drudgery of the statistical analyses by Mrs. Eleanor M. Brew.

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ON THE INCREASED URIC ACID CLEARANCE FOLLOWING THE INTRAVENOUS INFUSION OF HYPERTONIC GLUCOSE SOLUTIONS [1]

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We had observed previously that the uric acid clearance was not altered by the injection of 50 ml. of 50 per cent glucose (1). We concluded, therefore, that the active and maximal reabsorption of glucose had no effect upon the simultaneous reabsorption of uric acid. On the other hand, Talbot (2) has reported an increase in the amount of uric acid excreted when the blood glucose is maintained at relatively high levels (400 mgm. per cent) by a continuous infusion of glucose. In view of this apparent contradiction we have determined the uric acid and urea clearances during the continuous infusion of glucose. The results obtained confirm the correctness at least in part of both reports.

EXPERIMENTAL PROCEDURE

The subjects were normal women in the early puerperium. They were in the postabsorptive state. The procedure used is presented in Figure 1.

The glucose solutions administered intravenously were commercial preparations of 20 and 50 per cent glucose in distilled water.

Blood samples were obtained by venipuncture, usually from the cubital vein. The bladder was emptied by air injection and washing with saline whenever necessary.

Plasma and urine uric acid were determined by the Folin 1922 method slightly modified as previously described (1). Blood and urine urea were determined by the method of Van Slyke and Kugel (3). Blood sugar was determined by the Benedict blood sugar method (4). Urinary sugar was detected or quantitatively determined by the respective Benedict qualitative (5) or quantitative (6) methods.

The rate of creatinine excretion was used to check the completeness of the urine collections. Creatinine was determined by the Jaffe reaction by a procedure described elsewhere (7).

RESULTS

The rate of uric acid excretion and therefore the uric acid clearance is markedly increased when the blood glucose is maintained well above normal physiological values. The rate of excretion seems to be a function of the blood sugar level.

At the same time, the urea clearance and the rate of the creatinine excretion increase only slightly. This increase is of the same order of magnitude in both instances, and averages about 1.25 times the values observed during the control periods.

Representative results of a single experiment are presented in Table I. It will be noted that the uric acid clearances observed during the infusion of the glucose averaged 2.54 times greater than the clearances observed during the control periods. The urea clearances, however, averaged only 1.08 times greater during the infusion than during the control periods. An appreciable decrease in the plasma uric acid was observed only in this one instance. The decrease is not of sufficient magnitude, however, to account for the observed change in clearance.

The results obtained on the four subjects studied are given in Table II. The data are expressed as a ratio of the average values observed during the experimental periods to those obtained during the control periods. In each case the creatinine excretion and the urea clearance increase by approximately the same amount. The increase in the uric acid clearance, however, is significantly greater than the increase in the other two functions measured.
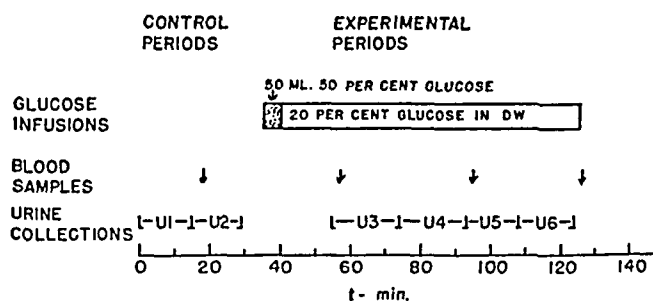
FIG. 1. EXPERIMENTAL PROCEDURE

TABLE I

Summary of data from subject O. V.   Surface area = 1.85 sq. m.

Period	Blood			Urine				Clearance	
	Glucose	Urea	Uric acid	Glucose	Urea	Uric acid	Flow	Urea	Uric acid
	mgm. per cent	*mgm. per cent*	*mgm. per cent*	*grams per cent*	*mgm. per cent*	*mgm. per cent*	*ml. per min.*	*ml. per min.*	*ml. per min.*
1	77	12.1	7.1	0	130	9.7	9.9	99	12.7
2	77	12.1	6.9	0	117	9.2	10.4	93	13.1
Ave.	77	12.1	7.0					96	12.9
Glucose Administered									
3	400	12.0	6.6	1.5	62	9.1	26.1	99	26.1
4	540	12.3	6.4	1.6	49	8.4	31.9	97	31.9
5	640	12.9	6.1	1.8	42	6.4	36.2	112	36.2
6	690	12.5	5.7	1.8	39	6.3	37.9	107	37.9
Ave.	566	12.4	6.2					104	33.0

It is probable that the factors which are operating to increase the urea clearance and the rate of creatinine excretion are also operating to increase the uric acid clearance. If it is assumed that the increase in the urea clearance and the creatinine excretion indicates an increase in the filtration rate, then part of the increase in the uric acid clearance must also be referable to this changed filtration rate. Even so, the increase in the uric acid clearance is still significantly greater than the increase in the other functions measured. It would appear, therefore, that this increase in the uric acid excretion is due not only to an alteration in the filtration rate, but also to an alteration in the tubular reabsorption of uric acid.

The maximum uric acid clearance which we have been able to obtain under these circumstances has been about one third of the normal inulin clearances.[2] To attain such values, the blood sugar levels were maintained between 600 and 1000 mgm. per cent.

DISCUSSION

These experiments show why we did not observe any changes in the uric acid excretion in the previous work (1). In those experiments the glucose was given in a single 50 ml. injection of 50 per cent glucose and the urines were then collected in three separate one-hour periods following the injection. Under such circumstances the blood sugar levels should have been high for at least 15 to 30 minutes following the administration of the glucose (8). The tubular excretory mass for glu-

[2] Taken to be about 120 ml. per min. per 1.73 sq. m.

cose would have been exceeded only during this time. Any alteration in the uric acid excretion due to the presence of glucose should have been seen during the first of the three periods. Since the uric acid excretion and therefore the clearance remained essentially constant under these circumstances, it was concluded that "the injection of hypertonic solutions of glucose exerted no effect upon the excretion of uric acid" (1).

This statement is still correct in part, since glucose injections or infusions affect the uric acid excretion only when the glucose level in the tubule reaches and is maintained at relatively high levels during the period of the urine collection. In other words, glucose in the kidney tubule at physiological levels (possibly with respect to both concentration and position) does not affect the uric acid reabsorption. The decrease in the rate of uric acid reabsorption occurs, then, only when the glucose in the kidney tubule is maintained at levels in excess of the ability of the tubule to reabsorb the glucose.

TABLE II

The ratio of values of the uric acid and urea clearances and the rate of creatinine excretion observed during the intravenous infusion of glucose to the values observed during the control periods

Subject	Uric acid clearance	Urea clearance	Creatinine excretion
O.V.	2.54	1.08	1.13
M.C.	2.08	1.25	1.25
S.T.	3.41	1.28	1.45
M.E.	2.26	1.21	1.27
Ave.	2.57	1.25	1.23

These data also confirm Talbot's results qualitatively but not quantitatively. He states (2) that it is possible to increase the amount of uric acid excreted by the infusion of glucose so that the uric acid clearance approaches the glomerular filtration rate. We, on the other hand, have not been able to obtain values greater than one-third of the normal inulin clearance as was indicated above. It is possible that this quantitative difference is due to a difference in the specificity of the methods used for the determination of uric acid.

These data indicate, therefore, that this effect of high tubular concentrations of glucose might be due directly to the presence of glucose which competes in some mechanism, or part of a mechanism, which is involved in the reabsorption of the uric acid. Or the effect may be due to the diuresis produced by the glucose, since the inhibition occurs only when a diuresis occurs.

However, a water diuresis does not produce such an increase in the uric acid clearance (1, 9) although it must be granted that urine flows as great as those produced by glucose cannot be produced readily by water alone. Moreover, there seems to be a better correlation between the blood glucose concentration and the uric acid clearance, than there is between the urine flow and the uric acid clearance. This might indicate that the inhibition of the reabsorption of the uric acid by the glucose is independent of the urine flow.

We have assumed that the observed increase in the urea clearance and the rate of creatinine excretion is due to an increase in the glomerular filtration rate. Contrariwise, Selkurt (10) has reported no increase in glomerular filtration in the dog receiving infusions of hypertonic glucose. Similarly, Klopp, Young and Taylor (11) did not observe any change in the glomerular filtration rate in man when glucose and para-aminohippuric acid at low plasma levels were infused, but they did observe an increase in glomerular filtration rate when the concentration of the para-aminohippuric acid was increased. They did not, however, try infusions of glucose.

These data do not yield any information as to the actual mechanism involved. The possible mechanisms by which such a phenomenon may occur have been discussed by Selkurt (10) who has observed that glucose at plasma levels similar to those employed in these experiments interferes with the reabsorption of ascorbic acid in the dog.

SUMMARY

The rate of uric acid excretion and therefore the uric acid clearance is markedly increased when the blood glucose is maintained well above normal physiological levels. The rate of uric acid excretion seems to be a function of the increase in the blood sugar level. With the blood sugar maintained at an average value of 499 mgm. per cent, the average uric acid clearance increased in four patients 2.57 times the control clearances. At the same time the urea clearance and the rate of creatinine excretion increased only 1.25 times the values observed during the control periods. Some of the factors which may be operating to produce these results are discussed.

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EDEMA AND DECREASED RENAL BLOOD FLOW IN PATIENTS WITH CHRONIC CONGESTIVE HEART FAILURE: EVIDENCE OF "FORWARD FAILURE" AS THE PRIMARY CAUSE OF EDEMA [1]

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During the course of chronic congestive heart failure it is known that patients retain salt and water. It seems evident that this is not due to forcing of fluid into the tissues by an increased hydrostatic pressure, since such a mechanism would result in hemoconcentration, whereas chronic heart failure produces hemodilution (1, 2). Furthermore, Warren and Stead have shown that in chronic congestive failure the weight gain and blood volume increase precede the rise in venous pressure (3). This fact points to a renal factor in heart failure, and the results of an investigation of this factor by some of the newer techniques (4) is the subject of this paper.

Patients were chosen with chronic congestive failure requiring mercurial diuretics at frequent intervals to maintain compensation. This group was selected because they tended to become edematous at bed rest when diuretics were withheld and, therefore, would be expected to show at bed rest whatever abnormality was responsible for their edema. Many patients with heart disease who have become decompensated when active show no abnormality of the circulation at rest. Such patients would therefore not be expected to show any disturbance in function leading to a retention of salt and water unless observations were made during exertion.

METHODS

Patients with hypertension or obvious renal disease are excluded from the discussion in the text but a few hypertensive individuals are included in Table I and Figures 1, 2 and 3 for comparison.

Because of orthopnea most patients required a 10 to 20 degree elevation at the head of the bed, and to rule out

this factor two subjects with failure were studied flat and at an elevation of 60 degrees. The renal blood flow and filtration rate, instead of falling as in a normal individual (5), remained the same.

The renal plasma flow and filtration rate were studied with sodium para-amino hippurate [2] and inulin as described by Smith et al. (4). Observations were made after a 6- to 8-hour fast. Each patient was given 2 glasses of water 30 minutes before, and phenobarbital 0.03 grams an hour before, the test. Priming doses of inulin and hippurate, calculated to give extracellular fluid and blood levels of approximately 2 mgm. per cent hippurate and 50 mgm. per cent inulin were administered. A sustaining venoclysis, which contained sufficient inulin and hippurate to maintain constant blood levels, was delivered at the rate of 4 ml. a minute. Forty minutes were allowed for equilibrium to be established between plasma and extracellular fluid. The bladder was then emptied through a catheter (4-hole when possible) by injection of 10 ml. of air and aspiration with a 50 ml. syringe. The air was reinjected, and urine and air expressed with pressure over the bladder. Then 20 ml. of distilled water followed by 30 ml. of air were introduced and aspirated. Finally, the air was reinjected and all possible residue expressed with bladder pressure alone. This entire maneuver occupied a period of one and a half minutes. Three or four 15-minute samples of urine were obtained and venous blood specimens were drawn at the mid-point. This blood was collected using metycaine as a local anesthetic since novocaine gives the same color reaction as hippurate with the coupling reagent. It was found that introducing a needle through a novocaine wheal yielded answers 4 or 5 times the true values. Patients receiving any sulfonamide preparation were excluded from the study because these drugs also contain the para-amino radical. The cells were separated from the plasma by centrifugation within an hour after collection of the blood. In man, we have not found it necessary to separate and precipitate the plasma immediately between collection periods, though Smith advises this in dogs because para-amino hippurate enters their red blood cells. Two ml. portions of plasma were precipitated by the cadmium sulfate-sodium hydroxide method and the plasma para-amino-hippuric acid level determined by a modification of the Bratton-Marshall technique for sulfonamides (6).

One ml. portions were precipitated with acid zinc sulfate, and inulin determinations were made by the method of Corcoran and Page (7). Urine was diluted in volumetric flasks to a urine to plasma ratio of approximately one. This was done in 2 steps, the first dilution being used for inulin determinations and the second for hippurate, starting with 5 to 10 ml. of urine measured from an accurate pipette. Standards for inulin and hippurate were made up from dilutions of the original ampules and were treated in the same manner as the unknowns. Standards were not made up from dried free acid and from dried inulin, since it is the urine to plasma ratio which determines the clearance, and exact quantitation is not necessary. All urine and blood specimens were run in duplicate and usually agreed within 2 per cent, with occasional variations of 4 per cent.

The gravimetric uranyl zinc acetate method as described by Butler and Tuthill (8) was used to determine the blood sodium. Each determination was run in duplicate and all duplicates at variance more than 2 m.eq. were discarded.

The cardiac output was determined in some of the patients usually within a few days of, and sometimes simultaneously with, the renal studies. The direct Fick principle was employed, utilizing a radiopaque ureteral catheter passed from the cubital vein to the right atrium under fluoroscopic control (9). Arterial blood was obtained from an inlying needle with a stylette in the femoral artery. The oxygen consumption was measured by the analysis of a 2-minute sample of expired air collected in a Douglas bag.

Venous pressures were estimated with a No. 19 needle connected to a glass manometer filled with normal saline, using a point 5 cm. below the 4th right costochondral junction for a base-line, and atrial pressures were measured from the same point. Measuring from the back was not feasible on our bed (10).

RESULTS

All results were corrected to a body surface area of 1.73 square meters to make them comparable. Thirty-nine studies on thirty-five normal individuals without evidence of renal disease or hypertension gave average figures of 626 ml. per minute per 1.73 square meters for the renal plasma flow, 123 ml. per minute for filtration rate, and 20.6 per cent for the filtration fraction. The mean value for renal plasma flow was 626 ± 165 ml. per minute per 1.73 square meters; for filtration rate, 129 ± 40 ml. per minute per 1.73 square meters excluding three patients who were far out of line. We did not find the filtration fraction (RPF/FR) so constant as did Goldring and Chassis (11), the mean being 20.6 ± 5.6 per cent. Eight patients had filtration fractions outside of 20 per cent ± 5. Two of the latter were studied

a second time with essentially the same results. It is possible that some of our patients were abnormal as many came from the older age groups. The wide variation of the renal plasma flow and filtration rate is partially accounted for by the fact that both males and females are included. The means of Goldring and Chassis were 697 ± 135.9 ml. per minute per 1.73 square meters renal plasma flow for males, and 594 ± 102.4 ml. per minute for females; 131 ± 21.5 ml. per minute per 1.73 square meters filtration rate for males, and 117 ± 15.6 ml. per minute per 1.73 square meters for females. The mean filtration fraction was 20 ± 0.03 per cent (11).

In the patients with chronic failure the renal plasma flow was reduced to one-third to one-fifth normal. The filtration rate was one-half to one-third normal, giving filtration fractions ranging from 30 to 50 per cent (Table I). The filtration fraction is obtained by dividing the filtration rate by the renal plasma flow. It represents the percentage of the renal plasma flow which is filtered. High values indicate a high filtration pressure, probably most frequently due to efferent arteriolar constriction (12).

Figure 1 shows the results of renal studies in cardiac failure on the same and different subjects at various venous pressures. There is no significant correlation between venous pressure levels and inulin and hippurate clearances. In many patients these studies were repeated after the venous pressure was lowered by mercurial diuretics. The renal blood flow remained low despite the change in venous pressure. An increase in renal blood flow in some patients was observed when digitalis was given, or when the cardiac output increased during the period of observation. For this reason a decrease in venous pressure was at times associated with a rise in renal plasma flow. Two patients with acute failure had a normal cardiac output at rest and a normal renal plasma flow despite a high venous pressure. These patients made a rapid recovery. The lack of correlation between the venous pressure and the renal blood flow is even more evident in these patients with acute failure.

Figure 2 shows the correlation between the renal plasma flow and the cardiac index. The cardiac index is the cardiac output per square meter of body surface area, and the normal range in our

TABLE I

Circulatory and renal studies in patients with heart failure

Date	Patient	Diagnosis	Sex	Age	Venous pressure	Atrial pressure	Arterio-venous O₂ difference	Renal A-V difference	Cardiac output per sq. m. = cardiac index	PAH clearance (See also reference 15)	Renal plasma flow	Filtration rate	F.R./R.P.F. =(Filtration fraction)	Hematocrit	Remarks
					mm. saline		*vol. per cent*		*liters per sq. m. per min.*	*per cent*	*per 1.73 sq. m. ml. per min.*		*per cent*		
5-24-45	M.B.	Hypertensive heart disease	M	35							121	63	52.0	54.2	
6-23-45	F.M.	Hypertensive heart disease	M	51	250 185		8.4 6.9		2.0 2.3		144 151	78 85	54.0 56.4	41.2 40.0	After Lanatoside C
6-30-44	W.R.	Hypertensive heart disease	M	29							274	128	46.7	37.3	
1-8-45	R.J.	Hypertensive heart disease	F	62	85						135	47	34.8	49.8	
8-15-44 9-5-44	E.R.S.	Hypertensive heart disease	F	53	70 45						195 315	101 101	51.8 32.2	39.6 33.5	
7-19-45 7-23-45	C.Mc.	Hypertensive heart disease	M	63		135 30 35	6.2 4.7		2.7 3.8	91	282 294 326	162 164 131	57.4 55.8 40.2	41.8 40.4 39.2	After Lanatoside C
6-26-45	J.McW.	Rheumatic heart disease	M	50	70		4.9	2.8	3.2		242	76	31.4	35.6	
12-29-44	F.L.R.	Rheumatic heart disease	F	44							308	53	17.3	39.8	
4-13-44	V.L.	Rheumatic heart disease—pulm. infarct.	F	13		200	7.4	2.5	2.23	92	252	111	44.0	39.9	
7-19-44 7-28-44	F.W.	Rheumatic heart disease	F	36	225 215						315 303	94 84	30.0 27.7	30.9 28.7	
10-5-44 10-12-44 10-19-44 5-29-45	L.F.	Rheumatic heart disease	F	36	95 95 150	105	5.3 6.3		1.9 2.4 2.3		291 348 206 248	56 51 48 57	19.3 14.7 23.3 23.0	29.8 31.4 25.5 31.4	
6-20-45 7-2-45	S.H.	Rheumatic heart disease	F	45	270	140	5.0	4.0	2.9	90	197 174	87 72	44.0 41.3	44.3 42.5	

TABLE 1—Continued

Date	Patient	Diagnosis	Sex	Age	Venous pressure	Atrial pressure	Arterio-venous O₂ difference	Renal A-V difference	Cardiac output per sq. m. = cardiac index	PAH clearance (See also reference 15)	Renal plasma flow	Filtration rate	F.R./R.P.F. = (Filtration fraction)	Hematocrit	Remarks
					mm. saline		*vol. per cent*		*liters per sq. m. per min.*	*per cent*	*per 1.73 sq. m. ml. per min.*		*per cent*		
5-14-45 * 6-8-45	L.H.	Rheumatic heart disease	M	41	195 136	170	6.4 6.2 6.8	4.4	2.3 2.7 2.1	64	148 166 177	60 79 64	40.5 47.5 36.2	41.1 40.0 37.2	After Lanatoside C
3-1-45	M.W.	Rheumatic heart disease	F	56							187	80	42.8	42.6	
2-6-45	P.W.	Rheumatic heart disease	F	62	165						229	80	34.9	46.3	
2-7-45	H.R.	Rheumatic heart disease	M	35	160						159	75	47.1	34.3	
7-11-45	J.M.L.	Rheumatic heart disease	F	34	210		8.1		1.5		193	93	50.8	40.0	
6-21-44 6-26-44	J.C.	Rheumatic heart disease	M	54		60	7.9		2.0		148 175	58 62	39.2 35.4	35.4 38.1	
9-20-44 10-2-44	G.E.	Rheumatic heart disease	F	55	140 145						110 162	54 71	49.0 43.8	50.7 47.8	
7-12-44	L.K.	Rheumatic heart disease—pulm. infarct.	F	31	Very high clinically	85	5.2	2.8	4.2	93	115	47	40.8	40.6	
7-14-45	W.R.	Syphilitic aortic insufficiency	M	45	45						332	96	28.9	36.7	
6-18-45 6-13-44	H.M.	Syphilitic aortic insufficiency and auricular fibrillation	M	35		130	7.0		2.0		254 130	78 58	30.7 44.6	43.3 46.8	Autopsy
3-27-45	G.H.	Syphilitic aortic insufficiency	M	57	40		3.2		4.0		169	71	42.0	45.7	
5-3-45 5-17-45	W.F.	Syphilitic aortic insufficiency	M	58	250 50						143 256	79 90	55.3 35.2	45.9 47.7	
1-10-45 1-18-45 6-6-45	A.H.	Syphilitic aortic insufficiency	F		160 75 65		5.3		2.4		200 136 93	60 35 45	30.0 25.7 48.3	39.6 39.9 41.7	

TABLE 1—*Continued*

Date	Patient	Diagnosis	Sex	Age	Venous pressure	Atrial pressure	Arterio-venous O₂ difference	Renal A-V difference	Cardiac output per sq. m. = cardiac index	PAH clearance (See also reference 15)	Renal plasma flow	Filtration rate	F.R./R.P.F. = (Filtration fraction)	Hematocrit	Remarks
					mm. saline	mm. saline	vol. per cent		liters per sq. m. per min.	per cent	per 1.73 sq. m. ml. per min.	per 1.73 sq. m. ml. per min.	per cent		
4-5-44	D.B.	Arteriosclerotic heart disease	M	46							222	72	32.4		
6-28-45	A.W.	Arteriosclerotic heart disease	M	58		220 170	9.5 7.9		3.2 3.8		158 212	91 94	57.6 44.3	47.0 44.5	After Lanatoside C
6-28-44	S.J.	Heart disease	M	33		160	7.0		2.4		140	65	46.4	36.7	
10-16-44	R.J.	Rheumatic heart disease	F	37							448 372	100 68	22.3 18.3	37.2	After Lanatoside C
4-21-45	T.S.	Rheumatic heart disease	M	54		215 155	7.9 4.8 4.9		1.6 2.5 2.9		112 176 224	48 55 70	42.8 31.2 31.2	39.3 38.1	After Lanatoside C
4-30-45					30										
1-23-45	N.G.	Cor pulmonale	F		145 127		3.4		2.9		165 254	60 61	36.4 28.0	54.6 43.0	After Lanatoside C
7-5-45	W.L.	Syphilitic aortic insufficiency	M	47	35		5.2	2.4	2.6	85	272	93	34.6	38.0	
7-10-45	L.V.E.	Syphilitic aortic insufficiency—subacute bact. endocarditis S.B.E. healed	M	41	55		5.9	2.8	3.3	89.5	264	99	37.5	36.8	
8-21-45											429	109	25.3	28.1	
8-10-45	J.G.	Rheumatic heart disease	M	30		215 165	9.6 8.7		1.3 1.5		151 170	91 83	60.5 49.0	43.0 40.8	After Lanatoside C
10-9-45	W.W.T.	?Arteriosclerotic or rheumatic heart disease	M	75		245	7.7		1.7		114	51	45.0	34.8	
3-30-15	J.C.	Syphilitic aortic insufficiency	M	32	155		7.9	4.4	1.7	63	89	35	40.0	35.0	
3-8-44	M.R.	Syphilitic aortic insufficiency	M	45			8.5	5.5 -	1.6	83.5	176	70	40.0	45.0	

Patients with normal filtration rates did not have chronic failure, and are included here and in Figures 1, 2 and 3 for comparison, but are not referred to in the context. The same applies to the patients with hypertensive heart disease who may or may not have had chronic failure. They are designated by an open circle (0) in the Figures.
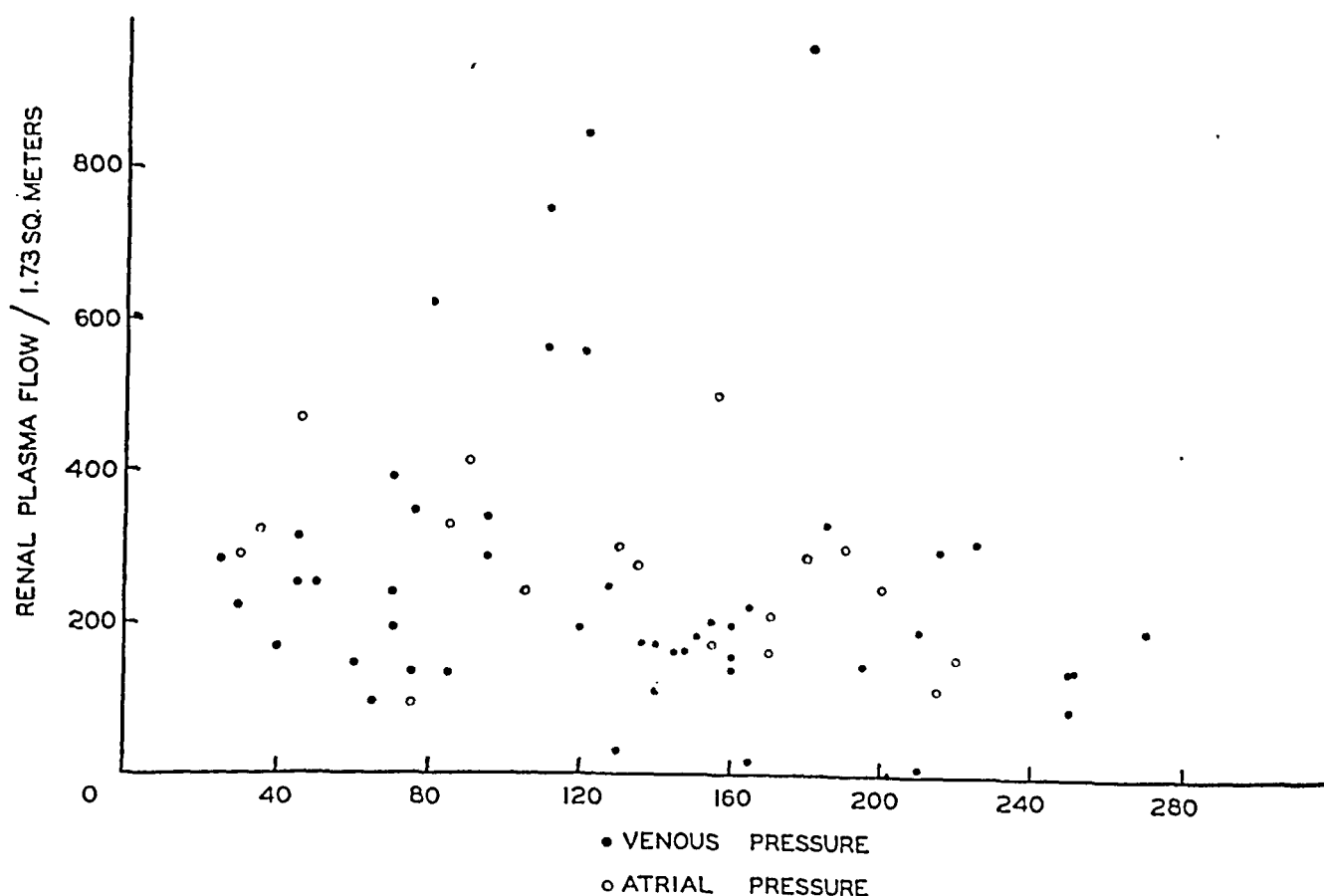
FIG. 1. RENAL PLASMA FLOW AT VARIOUS VENOUS PRESSURE LEVELS IN PATIENTS WITH CONGESTIVE
HEART FAILURE

Correlation coefficient r = − 0.1750. Pr is greater than 0.10, indicating no significant correlation.

laboratory is 2.3 to 4.1, with an average of 3.3 liters per square meter per minute (13). In general the renal plasma flow tended to fall as the cardiac index decreased. Two patients, G. H. and N. G., had a normal cardiac index with a low renal blood flow. It is possible that they had renal disease although this was not investigated.

From Figure 2 it is obvious that in many of the patients the absolute figure for the cardiac index is within the normal range. As pointed out previously there is no absolute level of the cardiac index below which patients develop cardiac failure (14). The cardiac index must be considered in relation to the metabolic needs of the subject. Thus patients with thyrotoxicosis may have cardiac failure with an output that is far above the normal resting level, but one which is inadequate for the increased metabolism. In many patients with cardiac failure and dyspnea the metabolic rate is increased. In these subjects the cardiac index may be in or above the normal range for resting subjects and heart failure still be present.

That the circulation was not optimum for the needs of our patients is shown by the increased arteriovenous oxygen differences. Figure 3 shows the renal blood flow plotted against the arteriovenous oxygen difference. In the great majority of the patients studied the arteriovenous difference was increased beyond the average normal value of 4 volumes per cent found in this laboratory (13), and that of 4.5 volumes per cent found in another laboratory (15).

The amount of sodium filtered was calculated from the product of the filtration rate times the blood sodium level. The amount of sodium excreted was calculated directly from the urine sodium, and the difference represents the amount resorbed. Twelve normals were studied and they showed an average value of 18.09 m. eq. sodium filtered per minute, 0.22 m. eq. excreted, and 1.23 per cent of filtered sodium excreted. In the patients with heart failure, one may see there was a definite decrease in the amount of sodium filtered, due to the low filtration rate (Table II). The
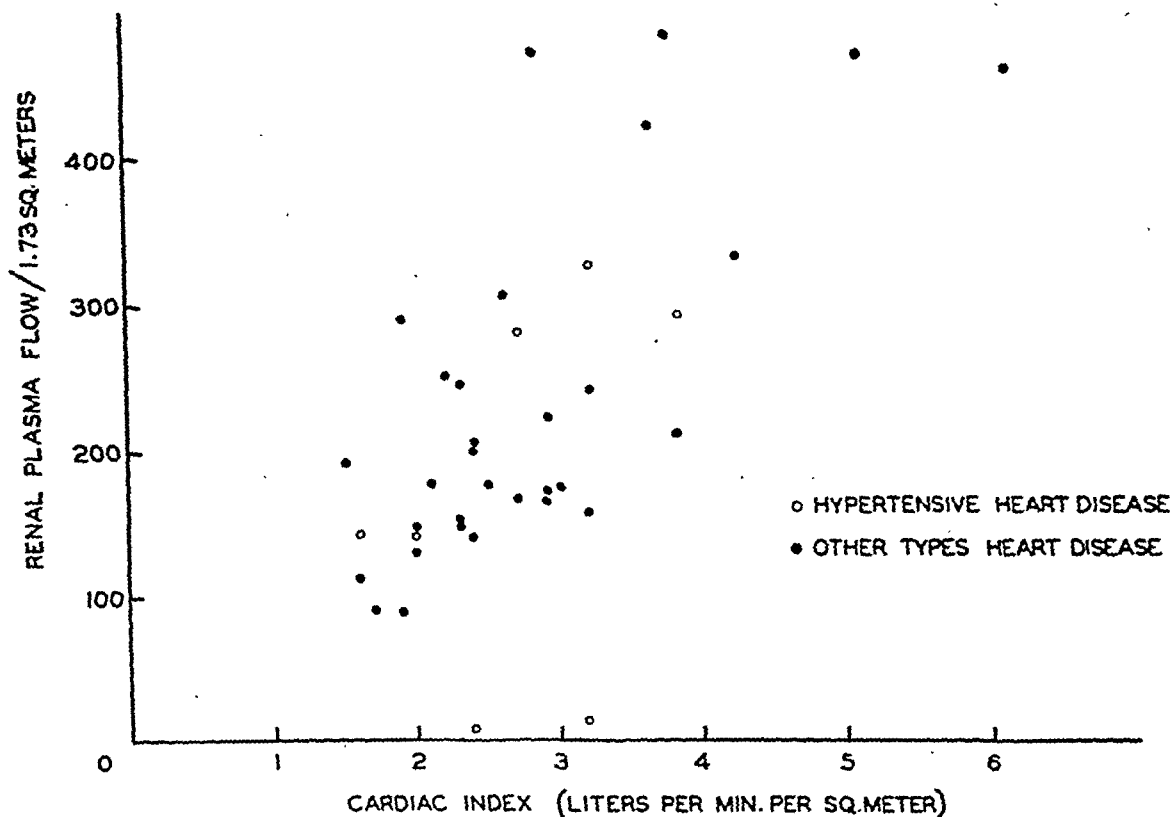
FIG. 2. RENAL PLASMA FLOW CORRELATED WITH CARDIAC INDEX IN PATIENTS WITH CONGESTIVE FAILURE
Correlation coefficient r = 0.6393. Pr is less than 0.10, indicating a high degree of correlation.

amount of sodium excreted per minute is also diminished. The percentage of the filtered sodium excreted is related to some interesting factors which bear upon the control of sodium excretion in normal individuals. Experiments clarifying these relationships have been done and will be reported in a separate communication.

DISCUSSION

The reduction in renal blood flow in patients with chronic heart failure is so marked that the question immediately arises as to whether or not the method is valid in heart failure. The method is dependent upon the almost complete removal of hippurate in a single passage through the kidney. To see if this occurred in heart failure, blood was obtained by passing a catheter into the renal vein of cardiac patients in whom the renal blood flow was being measured by the hippurate technique (16). The hippurate concentration in this blood was compared with that of the arterial blood collected simultaneously. The results in Table I indicate a normal clearance even in patients with severe heart failure. The calculations for the renal plasma flow were corrected to the average clearance figure of 88 per cent in the two cases in which the clearance was distinctly below normal. One of these, L. H., had severe pyelonephritis in addition to his heart failure. The other, J. C., died of multiple pulmonary infarctions. Autopsy showed only severe renal congestion and edema. As may be seen, two of the most severe cases of failure, M. R. and S. H., cleared normally. Seymour et al. (2) found normal renal tubular concentrating ability in patients with failure. Van Slyke et al. (17) showed that in shock the renal blood flow could be reduced to as low as 3 per cent without decreasing the percentage extraction of hippurate by the kidneys. They also demonstrated (17) that the kidney differs from muscles, brain and other tissues in that the arteriovenous oxygen difference does not rise proportionately as the renal blood flow falls. A reduction to 20 per cent of normal in the renal blood flow produces no
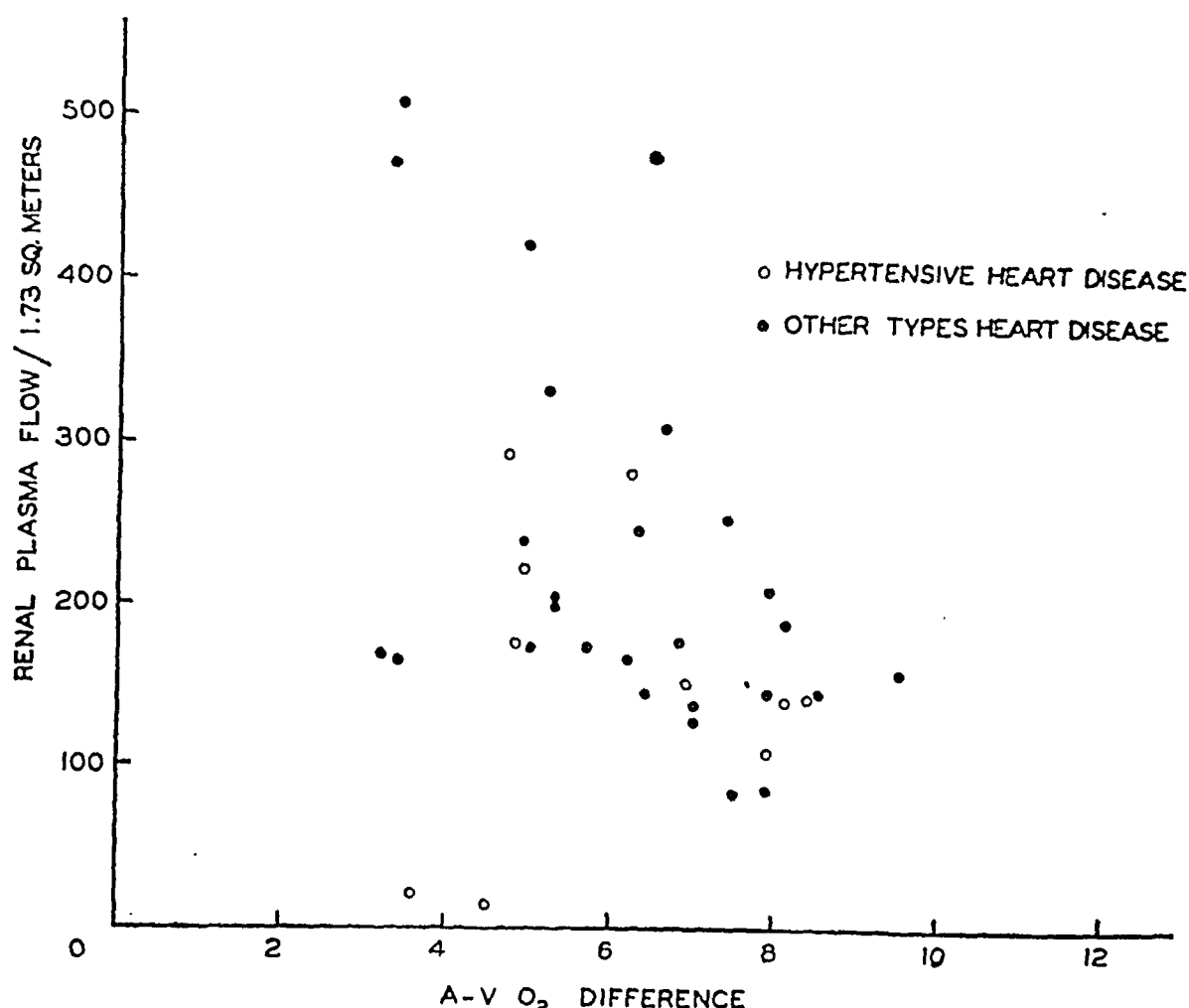
FIG. 3. RENAL PLASMA FLOW PLOTTED AGAINST THE ARTERIOVENOUS OXYGEN DIFFERENCE
Correlation coefficient $r = -0.4078$. Pr is less than 0.05 and greater than 0.02, indicating a significant correlation.

change in the amount of oxygen removed by the kidney. The renal blood flow of dogs in shock was reduced far out of proportion to the reduction in cardiac output, and they interpreted this as a saving mechanism to tissues more susceptible to anoxemia, such as the brain. The same mechanism seems to operate in patients with chronic congestive heart failure with a low cardiac output. In many of our patients the renal arteriovenous oxygen difference was increased. This is further evidence that the renal blood flow is actually markedly decreased, and that the low values are not the result of experimental error.

Our results are incompatible with the "backward failure" concept of edema formation in chronic heart failure, and point instead to a "forward failure" pathogenesis. In chronic congestive heart failure the low renal blood flow has no relationship to the height of the venous pressure

either in the same or different individuals. It can be correlated with an inadequate cardiac output. The cardiac output is rarely reduced below one-half the normal resting value, while the renal blood flow is frequently reduced to approximately one-fifth normal. This indicates a specific diversion of blood away from the kidneys, organs which with the subject at rest normally receive about 20 per cent of the cardiac output. The fact that the filtration rate remains relatively normal until the renal blood flow is markedly reduced suggests a high intraglomerular pressure from efferent arteriolar constriction (12). It is well known that renin, the renal pressor substance, causes efferent arteriolar constriction (18). By a technique previously described we have obtained blood directly from the renal vein in unanesthetized patients with chronic congestive failure (17). Preliminary bioassays (19) of this blood show a considerable

TABLE II

Excretion of sodium in patients with chronic heart disease

Date	Patient	Diagnosis	Renal plasma flow	Filtration rate	F.R./R.P.F. (Filtration fraction)	Sodium Amount filtered	Sodium Amount excreted	Excretion	Diuretics
			per 1.73 sq. m. ml. per min.	*per min.*	*per cent*	*m. eq. per min.*		*per cent*	
3-1-45	M.W.	Rheumatic heart disease	187	80	43.0	10.7	.153	1.4	Salyrgan 48 hrs. before
1-10-45	A.H.	Syphilitic aortic insufficiency	200	60	30.0	8.22	.08	.975	Salyrgan 1 week before
10-5-44	L.F.	Syphilitic aortic insufficiency	291	56	19.1				Salyrgan 1 week before
10-12-44			348	51	14.7	6.93	.05	.72	Salyrgan 4 days before
10-19-44			206	48	23.1	6.24	.02	.317	Salyrgan 11 days before
5-29-45			248	57	23.0	7.46	.10	1.34	Salyrgan 24 hrs. before
5-14-45	L.H.	Rheumatic heart disease	148	60	40.5	8.3	.0033	.039	
8-10-45	J.G.	Rheumatic heart disease	151	91	60.5	11.9	.0013	.011	

amount of renin (20). Increase in renin associated with a low cardiac output has been described in shock (21). A decrease in the amount of blood available to the kidney is supposed to be the fundamental cause of the increase in renin (22).

We attribute the salt retention, which in chronic congestive heart failure results in edema, to a low filtration rate which is caused by a marked reduction in renal blood flow. The data reported here demonstrate that the tubules continue to reabsorb salt at a fairly normal rate. The reason for this is not known, but it is probably related to their fundamental sodium-conserving mechanism. The decreased amount of sodium filtered in the presence of normal or slightly decreased reabsorption accounts for the fact that edema in cardiac failure develops with the quantity of sodium present in the average diet. Sodium retention will occur in normal subjects if the intake of sodium is greatly increased. Lyons showed that salt and water retention can produce a rise in venous pressure by administering large quantities of salt solution to normal people (23). Venous pressures as high as 170 mm. were observed. La Due found elevated venous pressures in some patients with acute glomerulonephritis without other evidence of heart failure (24). This was probably due to the low filtration rate produced by disease of Bowman's capsule (25).

Seymour *et al.* (2) found that the renal blood flow and filtration rate were decreased in patients with failure from hypertensive heart disease.

After digitalization and compensation of the patient, the renal blood flow rose while the filtration rate was relatively unchanged. They believed that the low renal blood flow and relatively high filtration rate during failure were due to the high venous pressure, because as compensation was restored the venous pressure fell and the renal blood flow increased out of proportion to the change in filtration rate. However, in each case which they report, a rise in cardiac output was present after compensation was restored by the use of digitalis, and it is to this that we attribute the rise in renal blood flow. The relatively high filtration rates were probably caused by efferent arteriolar constriction.

The evidence against "backward failure" or increased venous hydrostatic pressure as the cause of chronic cardiac edema is strong. It has been suggested that a slight rise of venous pressure which does not at first exceed the limits of normal produces the early edema in chronic heart failure. The fact that the gain in weight and the increase in blood volume precede a measurable rise in venous pressure indicates that a rise in capillary and venous pressures does not occur initially. A primary increase in venous and capillary pressures would cause hemoconcentration rather than hemodilution such as is found in chronic congestive failure.

Patients with acute heart failure associated with a sudden decrease in cardiac output may have a rise in venous pressure due to redistribution of

blood in the venous system produced by veno-constriction (26). The patients of Reichsman and Grant who had auricular fibrillation and were permitted to go into failure by omitting digitalis showed an initial rise in venous pressure followed by a gain in weight (27). They probably have a similar mechanism for their rise in venous pressure. Such subjects are not suitable for determination of the mechanism of sodium retention in heart failure, because a decrease in cardiac output and a rise in venous pressure occur simultaneously, and there is no means of telling which factor is responsible.

The low renal blood flow of patients with chronic heart failure might have resulted from pressure on the arterioles and veins from greatly swollen kidneys constricted by a tight capsule. In several patients with failure the venous pressure was reduced to normal with mercurial diuretics and kept at a normal value for several days. The renal blood flow remained essentially the same, demonstrating that a swollen kidney did not cause a reduction in renal blood flow.

Futcher and Schroeder (28) demonstrated the inability of the kidney to excrete salt properly in congestive failure by measuring the rate of excretion of salt in the urine after a venoclysis of concentrated salt solution. They found that cardiac subjects eliminated this salt much more slowly than normal controls. They attributed this to increased reabsorption of salt by the tubules. Our data on sodium excretion and reabsorption indicate that tubular reabsorption is not increased, and that the decreased output of sodium results from a decrease in the amount of sodium filtered, rather than an increase in the amount reabsorbed. These studies will be reported in detail elsewhere.

Several objections to the thesis that cardiac edema is caused by a reduction in renal blood flow arise. Many patients who are compensatable by bed rest alone, and who fail only with exertion, have a normal cardiac output and renal blood flow at rest (29). It is believed that these patients will be found to have an inadequate cardiac output and a low renal blood flow if they are studied under the conditions during which they developed the signs of heart failure; namely, during exertion or in the presence of infection. Studies on this problem are under way. A somewhat similar situation is found in patients with thyrotoxicosis, anemia

and beri-beri, who at rest may have failure with supernormal cardiac outputs. When the requirements for blood rise, as in anemia and thyrotoxicosis, the cardiac output may become inadequate for the increased demand for blood, even though the resting cardiac output still exceeds the value found in normal subjects. When the cardiac output is inadequate for the body needs, the kidneys, through a humoral or reflex mechanism, direct part of their blood supply elsewhere. Practically every patient with severe anemia has a decrease in renal blood flow, despite a marked rise in cardiac output (30, 31). In three patients with heart failure, two with anemia and one with thyrotoxicosis, a marked reduction in renal blood flow was found (28). In the thyrotoxic patient the renal blood flow rose to a supernormal value as the venous pressure fell and the edema disappeared. One of the anemia patients had a normal renal blood flow after recovery. The other did not, and was found to have renal disease.

Hypertensive patients who may have low filtration rates without edema are able to respond to exercise with an increase in cardiac output, and the shunting mechanism suggested above is not brought into play.

Patients with renal disease which has progressed to the point of uremia all have low filtration rates, many lower than any of our cardiac patients. Most of these individuals seen in this hospital during the past two years have had edema and/or an elevated venous pressure. Those without edema or elevated venous pressure have had nausea and vomiting and were unable to take salt and water. Hydration with salt and water intravenously promptly brought edema and elevated venous pressure.

Individuals with shock have very low filtration rates and yet rarely develop edema. The duration of shock is usually too short for the accumulation of salt and water. However, in prolonged shock edema may develop without other evidence of congestive heart failure (32).

The demonstration that salt and water are retained in patients with chronic cardiac decompensation on the basis of "forward failure" in no way invalidates the "backward failure" theory of pulmonary congestion. All of the phenomena observed on the ward are in accord with the concept that the left ventricle usually fails before the right. The assumption that this causes a rise in venous

pressure in the lungs seems acceptable. This rise in venous pressure may be responsible for the fact that fluid not excreted by the kidneys because of "forward failure" is deposited so early and extensively in the lungs. During the day the high capillary and venous pressures produced in parts of the body lying below the heart cause fluid to be forced into the tissues in the dependent portions of the body. It is believed that, when the patients lie down, this fluid is mobilized and is redeposited in the lungs because of the abnormally high capillary pressure from failure of the left ventricle. A reduction in renal blood flow is the primary cause of the retention of salt and water, but local changes in venous and capillary pressures produced by gravity and left ventricular failure determine where the retained fluid is deposited.

SUMMARY AND CONCLUSIONS

1. Patients with chronic congestive heart failure without evidence of hypertension or renal disease were studied to determine the rôle of the kidney in the formation of cardiac edema. These patients tended to have an inadequate cardiac output by the catheter method utilizing the direct Fick principle, and a low renal blood flow by the para-amino hippurate method.

2. The filtration rate by the inulin technique was reduced only about half as much as the renal plasma flow, frequently giving filtration fractions higher than those reported in any other condition. This indicates a high intraglomerular pressure, which was probably caused by efferent arteriolar constriction.

3. Studies of sodium filtration, excretion and reabsorption show that the retention of salt resulting in edema is caused by the low filtration rate, and is not due to increased reabsorption of salt.

4. The renal blood flow was reduced to about one-fifth normal, when the cardiac output was approximately half normal, indicating a specific diversion of blood away from the kidney.

5. It is suggested that a similar shunting of blood from the kidneys may be important in those patients who have a normal renal blood flow and normal cardiac output at rest, but who develop evidence of heart failure and edema on exertion. When the cardiac output becomes inadequate to meet the demands of exercise, blood may be diverted from the kidneys to other parts of the body whose metabolic needs are greater.

6. The reduction in renal blood flow had no relation to the venous pressure, but was correlated with the reduction in cardiac output, indicating that it is a "forward failure" phenomenon and not due to "backward failure" or increased hydrostatic pressure in the veins.

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THE EFFECT OF SIGNIFICANT WEIGHT CHANGE ON THE PREDICTED PLASMA VOLUME

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With the introduction of improved dye methods for determining the plasma volume, numerous clinical studies have been made in various conditions. It has been found that the plasma volume is influenced by age, sex, height, weight, muscularity (1), position, exercise (2 to 5), seasonal and climatic factors (6), as well as by different disease states.

Gibson and Evans concluded that normal values are best estimated from surface area measurements if no marked disturbance in weight-to-height relationship exists, and from height in those cases presenting weight changes due to disease (1). Repeated observations have shown that the plasma volume usually approximates 1600 ml. per square meter of body surface area. It has also been suggested that for practical estimation, the plasma volume may be calculated from body weight, using the value of 45 ml. per kgm. (7).

The present study was undertaken because of the marked discrepancies noted on occasion between actual and estimated plasma volume measurements, based on surface area, raising the question as to the effect of significant weight change on the predicted values.

MATERIALS AND METHODS

Five underweight and five obese patients were studied either on the wards of the Presbyterian Hospital or in the Vanderbilt Clinic. In addition, three patients were studied before and after significant weight change. One (M. H.) was a hospital patient with uncomplicated and afebrile active pulmonary tuberculosis at the right lung apex, who gained seven kgm. in weight on a high caloric diet. The second (E. S.) was an obese ambulatory patient with mild but asymptomatic hypertension, no signs of cardiac insufficiency, who lost eighteen kgm. on a 1200 calorie diet without fluid or salt restriction. The third patient (C. G.) lost twenty-eight kgm. in association with widespread metastatic carcinoma of the prostate.

Patients having acute infection, cardiac insufficiency, renal disease, liver disease, hypoalbuminemia, endocrine or metabolic disorders, anemia, dehydration or fever were not included in this report.

Blood samples for hematocrit, serum protein and volume measurements were obtained with the patient lying flat after at least a twenty-minute period of inactivity in that position. The plasma volume was determined with the blue dye T.1824, the optical density being measured with the photoelectric colorimeter (8), using a serum sample drawn ten minutes after the injection of the dye (7). Predicted plasma volume values based on surface area were arbitrarily calculated on the basis of 1600 kgm. per square meter, while predictions based on height were determined from the data of Gibson and Evans (1). The difference between observed and predicted values was expressed as a percentage deviation.

RESULTS

Based on surface area, the plasma volume in five underweight patients (Table I) was invariably higher than predicted, the deviation being ten

TABLE I

Comparison of estimated and predicted plasma volume determinations in five underweight patients

Case	Sex	Height	Weight	Surface area	Hematocrit	Plasma volume	Predicted plasma volume		Deviation from predicted plasma volume	
							Based on surface area	Based on height	Based on surface area	Based on height
		cm.	*kgm.*	*sq. m.*	*per cent cells*	*ml.*	*ml.*	*ml.*	*per cent*	*per cent*
1	F	141	46	1.32	39	2320	2112		+10	*
2	F	163	46	1.47	38	2480	2352	2504	+5	−2
3	M	174	63	1.76	44	3520	2816	3090	+25	+14
4	M	150	52	1.46	40	3000	2336		+29	*
5	M	159	44	1.42	42	2660	2272	2550	+17	+4

* Data insufficient for volume prediction on such short patients.

TABLE II

Comparison of estimated and predicted plasma volume determinations in five obese patients

Case	Sex	Height	Weight	Surface area	Hemato-crit	Plasma volume	Predicted plasma volume		Deviation from predicted plasma volume	
							Based on surface area	Based on height	Based on surface area	Based on height
		cm.	*kgm.*	*sq. m.*	*per cent cells*	*ml.*	*ml.*	*ml.*	*per cent*	*per cent*
1	M	173	78	1.92	45	3020	3072	2986	−2	+1
2	F	158	81	1.83	42	2080	2928	2250	−29	−7
3	F	165	72	1.79	40	2360	2864	2436	−18	−3
4	M	179	90	2.09	46	3060	3344	3067	−8	0
5	F	169	69	1.79	41	2500	2864	2430	−13	+3

per cent or greater in all but one. In five obese patients (Table II) the reverse was true, all showing somewhat smaller volumes than predicted, three with more than a ten per cent deviation. On the basis of height alone, the percentage deviation of determined volume from predicted volume was not significant.

With reference to the three patients studied before and after change in weight (Table III), the one with seven kgm. weight gain (M.H.) exhibited a decrease in plasma volume, despite a six per cent rise in surface area and predicted values. The other two (E.S. and C.G.) showed practically no change in plasma volume after an eighteen and a twenty-eight kgm. weight loss respectively, although the surface area and hence the predicted volume measurement decreased by ten per cent in one and by twenty-one per cent in the other. No significant changes in hydration, serum protein or red blood cell concentration took place during the period of observation.

DISCUSSION

It has already been pointed out that muscular persons may have relatively more and obese individuals less blood per unit of body weight than those of normal habitus (1). Gibson and Evans suggest that the varying proportions of blood in such tissues as muscle and fat may account for this difference.

The evidence presented in this paper confirms the fact that marked deviations from normal in weight, and therefore in surface area, do not always cause a parallel fluctuation in plasma volume. Not only do thin and obese persons tend to have a plasma volume more closely approximating that of persons of average weight, but significant changes in weight in individual cases are not accompanied by proportionate changes in the plasma volume. In situations associated with marked disturbance in weight to height relationship, as pointed out by Gibson and Evans, predicted values

TABLE III

Comparison of estimated and predicted plasma volume determinations in three patients before and after significant change in body weight

Patient	Sex	Age	Date	Height	Weight	Surface area	Hema-tocrit	Hemo-globin	Serum proteins	Plasma volume	Predicted plasma vol. based on surface area	Change in plasma volume	Change in predicted plasma volume
				cm.	*kgm.*	*sq. m.*	*per cent cells*	*grams per 100 ml.*	*grams per 100 ml.*	*ml.*	*ml.*	*per cent*	*per cent*
M.H.	F	27	8-13-45	160	43	1.41	41	12.8	6.1	2540	2256	−7	+6
			10-24-45	160	50	1.50	43	14.2	6.4	2360	2400		
E.S.	F	52	8-10-45	164	82	1.89	45	13.6	7.2	2720	3024	−1.5	−10
			10-19-45	164	64	1.70	44	14.0	6.9	2680	2720		
C.G.	M	62	6-8-45	175	78	1.94	47	12.2	6.1	2940	3104	+5	−21
			1-4-46	175	50	1.60	44	11.6	5.8	3100	2560		

based on height appear to afford the most useful estimate of normal.

It is therefore apparent that clinical studies of the plasma volume may be in error if the underlying disorder is preceded or accompanied by any marked degree of weight loss or emaciation and if weight or surface area are employed in predicting the normal. Similarly, studies of alterations in the plasma volume in given disorders may lead to erroneous conclusions if the patients involved are either abnormally thin or obese unless some other basis of comparison than weight is used.

The range of variation encountered in normals (1), coupled with the many constitutional and environmental factors known to influence the plasma volume, combine to make predicted volume measurements rough approximations at best. In the presence of significant weight change in either direction, it is suggested that height or ideal weight figures be used in the calculation, taking into consideration the habitus of the patient.

CONCLUSIONS

1. Plasma volume determinations in five underweight patients were found to be higher than predicted values based on surface area, whereas in five obese individuals the reverse was true; much closer approximation was obtained when height was used in the prediction of normal values.

2. Weight loss and weight gain in three patients studied were not accompanied by proportionate changes in plasma volume.

3. In the presence of significant weight loss or obesity, it is suggested that height or ideal weight be employed to predict the normal plasma volume.

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SERUM IODINE IN HYPERTHYROIDISM, WITH PARTICULAR REFERENCE TO THE EFFECTS OF SUBTOTAL THYROIDECTOMY [1]

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The concentration of precipitable [2] iodine in serum is a sensitive index of concentration of circulating thyroid hormone (1 to 7). The level of precipitable iodine is characteristically elevated in untreated hyperthyroidism (1 to 3, 6) and tends to fall after iodine medication and after thyroidectomy (3). In the present study the changes following treatment were analyzed with special reference to the development of abnormally low or high levels of precipitable iodine at various intervals after operation.

MATERIAL AND METHODS

Whenever possible, total or precipitable iodine concentration in serum was measured before any treatment was given. Iodine medication in the form of strong solution of iodine U.S.P., 5 drops 3 times a day, was then given until the day of subtotal thyroidectomy, when it was discontinued. In a few instances the measurement of serum precipitable iodine concentration was repeated just before operation. At various intervals after subtotal thyroidectomy the concentration of iodine in serum was again determined. In many instances, including all those in whom the subtotal thyroidectomy was done prior to 1940, postoperative determinations alone were made. Only patients in whom the diagnosis of hyperthyroidism was established by a characteristic clinical picture and by subsequent pathological examination of the gland removed at operation are included. Basal metabolic rate and concentration of cholesterol in serum were also followed postoperatively.

In the years during which these observations were made (1940 to Oct., 1944) the technical character of the operation of subtotal thyroidectomy in the New Haven Hospital was planned by one of the authors (K. W. Thompson). The majority of the operations were performed by him, or done under his direction by the resident

surgeon. A rather complete standardized thyroidecto was carried out in the belief that a radical operation w reduce the incidence of recurrent hyperthyroidism, w is regarded as a highly unfavorable outcome. About operations for subtotal thyroidectomy were perfor during this period. An initial determination of the se total or precipitable iodine was made in 87 instances, of the whole blood iodine in 26 more. At some during the first 6 months after operation the se iodine determination was repeated one or more time 60 of these cases, which were selected more or les random. After 6 months serum iodine determinat were made in only 32 of these cases.

There were included also 51 additional cases wh operation had been done before 1940; in about hal these cases the subtotal thyroidectomy had been don the New Haven Hospital before 1940. Unlike the stu during the first 6 postoperative months, many of t later studies were made because of suspected hypo roidism or recurrent hyperthyroidism.

Analyses of serum precipitable iodine were made by method of Man et al. (3), adapted to the procedure Riggs and Man (8) for whole blood iodine using manganate acid ashing. Some values of the serum t iodine are included; normally in the absence of iod medication these do not exceed the corresponding cipitable values by more than one gamma per cent (In a few instances (those analyses made prior to Ma

[2] The "precipitable" iodine includes all iodine which is precipitated along with the serum proteins. It is the same fraction which is sometimes referred to as "protein bound" or "hormonal" iodine.

[3] The procedure was as follows: the anesthesia basal avertin, supplemented by cyclopropane and l procaine (1 per cent without epinephrine). In the jority of the cases the parathyroid glands, especi those at the lower poles, and the recurrent laryng nerves were visualized, in order to avoid their inju The right lobe of the gland was totally removed in cases except those in which it was unduly adherent or not separate easily from the recurrent nerve; when t occurred a thin fringe of gland was allowed to rema All of the lingula and the isthmus were removed togeth with the left lobe, save for a small piece of the left l lying over the recurrent nerve. The amount of gla allowed to remain measured approximately 2 × 1.5 × cm. There were usually small fringes of gland of ve small size at the upper and lower poles because the po vessels were usually divided at the level of the capsu of the gland.

1941) serum total iodine values were calculated from whole blood values by dividing the latter by the factor 0.6 (5). Cholesterol was determined by methods previously described (10).

RESULTS

I. Concentration of iodine in serum before treatment [4]

The total iodine concentration was determined in sera from 50 untreated cases of hyperthyroidism, and the precipitable iodine concentration in sera from 37 other untreated cases. The confidence limits indicated that 95 per cent of the values might be expected to fall between 28.74 and 6.63 gamma per cent and 90 per cent between 25.48 and 7.47 gamma per cent. In the actual distribution 3 values fell below 8.4 gamma per cent (5.8, 4.9, and 7.0) and 3 values above 25.0 gamma per cent (30.1, 36.5, and 55.1). The 2 lowest values were from patients with mild hyperthyroidism, who may have been in spontaneous remission, but who nevertheless came to operation within a few weeks. The 3 high values were found in cases of severe hyperthyroidism which were not otherwise remarkable.

A positive correlation existed between concentration of iodine in serum and basal metabolic rate in this series ($P < 0.01$), but it was not close.

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[4] The authors are indebted to Miss Barbara Bartels and Dr. Chester Bliss for this statistical analysis. Although the frequency distributions of the arithmetic values of both precipitable and total sets were somewhat askew, the distribution of their logarithms appeared normal by a graphic test. The logarithmic values were therefore subjected to statistical analysis, and the final results translated back into arithmetic terms. The mean value ($\bar{x}$) for concentration of total iodine was 13.76, standard error of the mean ($s\bar{x}$) ± 2.26 gamma per cent; the mean value for concentration of precipitable iodine was 13.85, $s\bar{x}$ ± 2.63 gamma per cent. (The standard errors were calculated by an approximate formula.) Because of a good agreement between the variances of the two sets (as tested by Bartlett's chi square test) and because of the good agreement of the means themselves, the entire 87 values could be pooled for estimation of confidence limits. The mean value for the entire group was 13.80, $s\bar{x}$ ± 1.71 gamma per cent. The confidence limits were calculated by the approximate formula $\bar{x} \pm t s \sqrt{\dfrac{N+1}{N}}$, where $\bar{x}$ is the mean, t (for the desired level of probability and the degree of freedom) is derived from Fisher's Tables, s is the standard deviation, and, N is the number of values in the series.
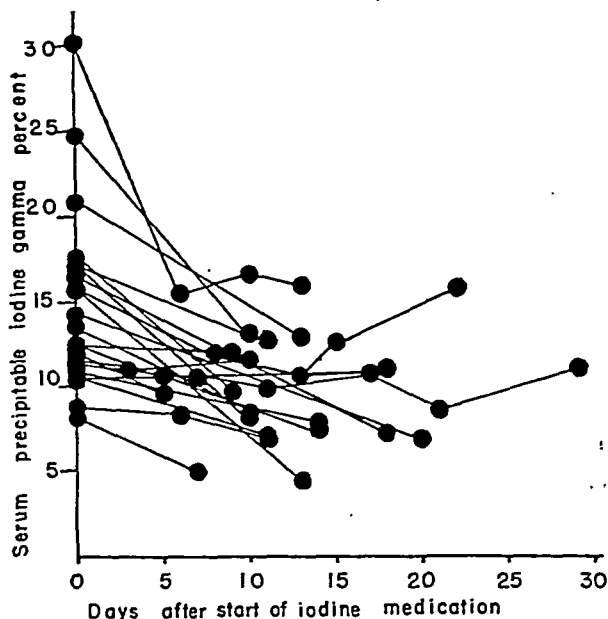
FIG. 1. EFFECTS OF IODINE MEDICATION ALONE (15 DROPS DAILY OF STRONG SOLUTION OF IODINE, USP) ON THE SERUM PRECIPITABLE IODINE IN 21 CASES OF PREVIOUSLY UNTREATED HYPERTHYROIDISM

Serum precipitable iodine tended in many cases to fall, especially when the initial concentration was high, but occasionally was unaffected. Normal concentrations (3 to 8 gamma per cent) were reached only in a minority of cases. Data from 5 of these cases have been published elsewhere (1).

Since the basal metabolic rate was usually determined only once before treatment, these values recorded probably often exaggerated the true pre-operative levels. Although this artifact was undoubtedly a factor tending to obscure a significant relationship, the dissociation between concentrations of serum iodine and of basal metabolic rate was often too gross to be readily attributable to this source. Also, high concentrations of iodine were not infrequently found in association with relatively slight elevations of the metabolic rate.

II. Effects of iodine medication alone

In Figure 1 are shown the results of iodine medication pre-operatively on the serum precipitable iodine of 21 previously untreated cases of hyperthyroidism. In general the concentrations tended to drop, especially in those cases in which it was initially rather high. In some instances, however, no distinct change occured. Within the second or third week period studied, the serum
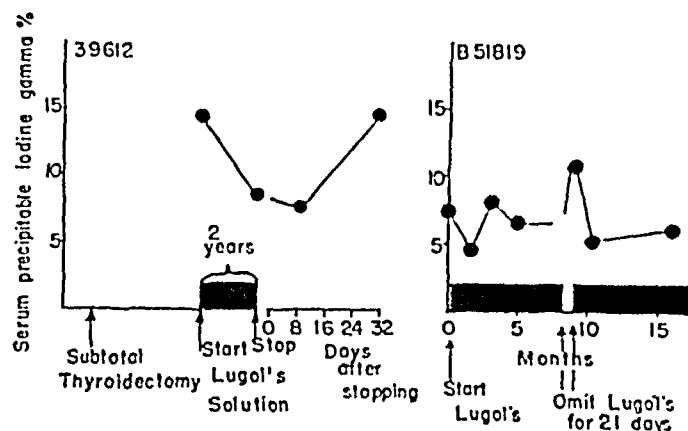
Fig. 2. Effects of Prolonged Iodine Medication (15 Drops Daily of Strong Solution of Iodine, USP) on the Serum Precipitable Iodine in 2 Cases of Hyperthyroidism

Partial (case 39612) or complete (case B51819) remission of hyperthyroidism was produced and maintained over many months by continued iodine medication, but interruption of iodine medication was promptly followed by a rise in serum precipitable iodine and clinical evidences of increased thyroid overactivity. Neither case, therefore, had become refractory or indifferent to iodine medication. Case 39612 was one of recurrent hyperthyroidism and subsequently underwent a second subtotal thyroidectomy; case B51819 has remained in complete remission with continued iodine medication after the brief interruption shown in the figure.

precipitable iodine occasionally fell to normal levels (3.0 to 8.0 gamma per cent), but more commonly remained somewhat elevated. Changes in basal metabolic rate paralleled those in the serum iodine; no distinct time lag could be detected.

Figure 2 illustrates the responses in two cases of hyperthyroidism in which iodine medication was continued for months and years. In the first case (*39612*), partial, in the second (*B51819*), complete remissions were maintained as long as the iodine medication was continued, but exacerbations of the hyperthyroidism promptly developed as soon as the iodine medication was discontinued.

III. Effects of subtotal thyroidectomy

(*A*) *Immediate effects.* In 4 cases the serum precipitable iodine was determined 3 hours after the conclusion of the operation and again at intervals during the next 2 weeks (Figure 3). After a slight rise on the day of operation the serum preciptable iodine fell, abruptly at first and then more slowly. Within 2 weeks the serum precipitable iodine of 1 patient (*A42903*)

had already fallen below normal levels. No symptoms suggestive of myxedema were present in this case. Of the other 3 whose levels were normal at this time, 1 (*A66295*) developed subnormal values some weeks later along with clinical changes necessitating thyroid medication.

(*B*) *Effects during the first half year.* In Figure 4 all the serum iodine values obtained during the first 6 months following subtotal thyroidectomy are plotted against time. During the first 2 weeks supranormal values (above 8.0 gamma per cent) are common, but after this interval only one such value appears. *This single high value* was associated with a clinical recurrence of goiter and hyperthyroidism, the only one in this series to appear within the first 6 months.[5] Subnormal values (below 3.0 gamma per cent) first appear at 14 days, then become and remain quite common after 3 weeks. Such abnormal values are only found in certain patients (about one quarter of the number studied), and are sometimes transient (Table 1).

(*C*) *Effects after the first half year.* In Figure 5 are collected all serum iodine values obtained in patients whose thyroidectomies had been done more than 6 months previously. In the group followed more than 5 years are included some cases in which the original operation had been done eleswhere years ago. Determinations of serum iodine after the first 6 months were usually obtained because of some clinical suspicion of overactivity or underactivity of the thyroid gland. Had all cases undergoing subtotal thyroidectomy here been called in and systematically examined, the proportion of normal values would doubtless have been greatly increased. Nevertheless Figure 5 does illustrate the frequency with which subnormal values for the serum iodine are found years after subtotal thyroidectomy. The cases having low concentrations of serum iodine in Figure 5 are analyzed more fully in Table I.

The occasional high values appearing in Figure 5 were all associated with clinical recurrence, usually mild, of hyperthyroidism. Seven recurrences developed before 5 years, 6 of which were so

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[5] This case was operated upon the first time by one of the residents early in his tenure, and there is some reason to believe that the procedure recommended was not carried out in its entirety.
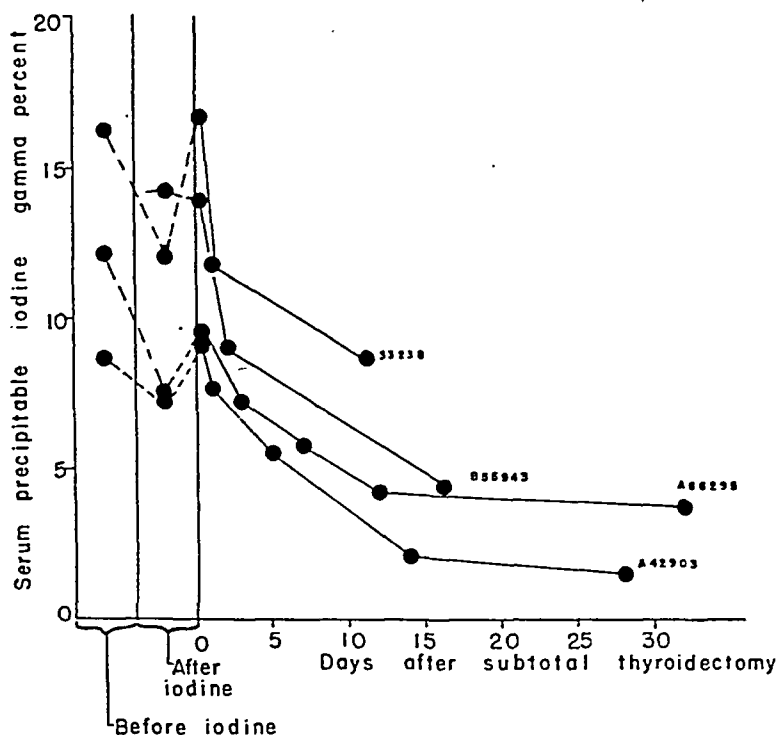
FIG. 3. CHANGES IN THE SERUM PRECIPITABLE IODINE IN 4 CASES OF
HYPERTHYROIDISM IMMEDIATELY FOLLOWING SUBTOTAL THYROIDECTOMY

Fifteen drops daily of Strong solution of iodine, USP, had been given prior
to operation but were discontinued on the day of operation. The first values
obtained 3 hours after the completion of the operation are only slightly higher
than the corresponding preoperative values. Iodine concentration fell during
the next few days, abruptly at first and then more slowly. In one case
(A42903) the concentration had become definitely subnormal within 2 weeks,
and remained depressed for as long as the patient was followed without thy-
roid medication (several months). One other case (B55943) developed a
subnormal level 3 to 4 months later; the other 2 (33238 and A66295) re-
mained within normal limits as long as they were followed (Table 1).

mild that they could be controlled by iodine medi-
cation alone without· operation. Only 1 case of
this entire group operated on and followed since
1940 has required a second thyroidectomy, and
only 2 cases of the approximately 130 cases op-
erated on in the previous 5-year period (1935
to 1940) have required a second operation after
1940. The occasional high values obtained in the
cases operated on here and elsewhere prior to 1940
illustrate the fact that the disease may recur after
a long interval of quiescence following subtotal
thyroidectomy, or it may smolder for many years
at levels of low intensity.

(D) *Subnormal values in individual cases.*
These were found at some time or another after
operation in 33 patients. The time of develop-

ment of these subnormal values and the associated
changes in basal metabolic rate, serum cholesterol,
and clinical status are all summarized in Table I
(their general distribution has already been shown
in Figures 4 and 5). In at least 3 instances
(*A45443, 95767,* and *B43492*) the low values
disappeared within 6 months. On the other hand,
there were 14 cases in which low iodine values
were present after more than a year, sometimes
after many years, and 3 more cases (*A35428,
B22457,* and *A42903*) in whom low values per-
sisted for at least 9 months. It is impossible
as yet to determine whether the remaining 13 cases
are to be classed as permanent or transient.
Twelve were followed for less than 10 months
before thyroid therapy was commenced or the pa-
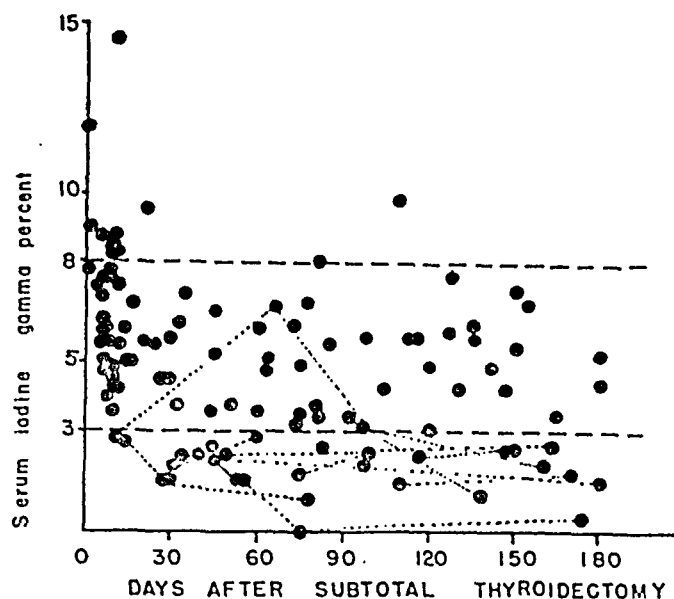
FIG. 4. EACH POINT CORRESPONDS TO ONE OF 116 INDIVIDUAL DETERMINATIONS OF SERUM IODINE IN 60 CASES OF HYPERTHYROIDISM DURING THE FIRST 6 MONTHS FOLLOWING SUBTOTAL THYROIDECTOMY

Dotted lines join repeated determinations of subnormal values in the same case. In the majority of cases the serum iodine concentration became and remained within normal limits after the first 2 weeks. High values were common during the first 10 to 15 days, and were only seen once after this interval. Subnormal values first appeared after 2 weeks and were frequently found after one month; many persisted throughout the entire period (Table 1).

tient disappeared from observation. The thirteenth case, *B32017*, maintained a level of serum iodine just about the lower limits of normal for about 2 years. Partial recovery of serum iodine levels soon after operation does not necessarily exclude later development of a chronic deficiency (*B7257*).

The basal metabolic rate in this group tended to be depressed, but in many instances a low level of serum iodine was associated with a basal metabolic rate which fell well within the normal range. For example, the minimum basal metabolic rate at the time of the low iodine fell below − 30 per cent only twice; it fell between − 30 and − 20 per cent in 8 cases, and between − 30 and − 10 per cent in 12, it was − 10 per cent or greater in 9 others (Table I). The serum cholesterol tended to be elevated, but exceptions were numerous. In the 24 cases studied, values exceeding 300 mgm. per cent were found in 13 instances, values below this level in the 11 others.

Sometimes the low levels of serum iodine were associated with outspoken clinical evidences of hypothyroidism, but more often this was not the case. The full-blown picture of sodden myxedema was rarely encountered. Necessarily, the rating of severity of the clinical signs and symptoms of hypothyroidism as 0, 1 +, 2 +, and 3 + in Table I is only the roughest sort of estimate. A rating of 1 + means simply minimal signs of thyroid deficiency, such as excessive weight gain; a diagnosis of hypothyroidism would hardly have been suggested except for the history of a subtotal thyroidectomy. A 2 + rating means minimal changes only in the facies or skin, and a fairly distinct response to thyroid medication. All cases with unmistakable facies, voice and hair changes, and psychomotor retardation were classed as 3 +. Only 5 cases exhibited changes marked enough to be classed as 3 +. In 10 of the 33 cases the clinical evidences of hypothyroidism never became more intense than 2 +, and in 13 more never exceeded 1 +. In 5 cases no clinical evidence at all of hypothyroidism could be detected. Almost all the cases rated as 1 + or more improved markedly in their sense of well being and experi-
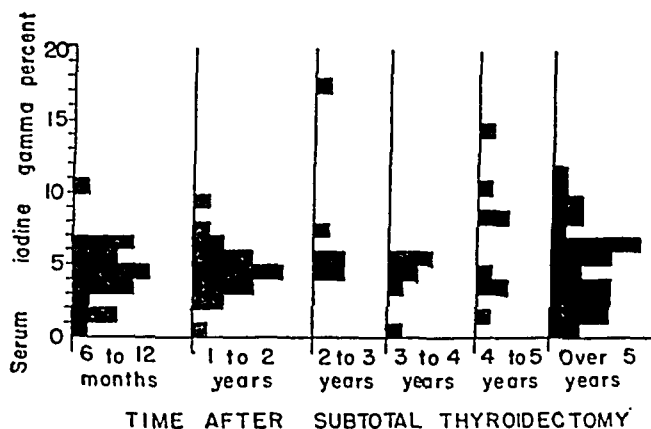
FIG. 5. EACH SMALL SQUARE CORRESPONDS TO ONE OF 97 DETERMINATIONS OF SERUM IODINE MADE MORE THAN 6 MONTHS AFTER SUBTOTAL THYROIDECTOMY IN 83 CASES OF HYPERTHYROIDISM

These determinations were usually made only in those cases in which there was clinical suspicion of hyperthyroidism or hypothyroidism, so that the proportion of abnormal values is undoubtedly much higher than would have been the case had all the patients been represented. The figure indicates, however, both the occasional recurrence of high values long after the initial operation, and the considerable number of cases with low values years after the operation (Table 1).

enced a sharp drop in the body weight with thyroid medication in doses not exceeding 0.06 grams daily. This is evidence that they were truly suffering from a state of partial thyroid deficiency, even though initially they were subjectively unaware of any disturbance.

DISCUSSION

It is commonly believed that subtotal thyroidectomy effects a complete and lasting cure of hyperthyroidism in the great majority of patients (11). The rarity of supranormal values of the serum iodine during the months and years follow-

ing subtotal thyroidectomy supports this view. It is also, however, generally held that permanent hypothyroidism following subtotal thyroidectomy is a rare occurence, much rarer, in fact, than recurrence of hyperthyroidism (12, 13). This latter belief may be seriously questioned in view of the considerable number of instances in which low values of the serum iodine are found long after the operation. The immediate sequel of operation in this series seems to have been hypothyroidism in many cases. Subsequently this disappeared in some cases while in others it persisted. Presumably this difference in behavior depended on dif-

TABLE I

Serum iodine, basal metabolic rate, and serum cholesterol at different intervals following subtotal thyroidectomy. All patients in whom the concentration of iodine in serum fell below 3.0 gamma per cent at any time after operation are included. Values obtained during or soon after a course of thyroid medication are omitted.

Number. Age in years. Sex	Time after operation	Serum iodine concentration†	BMR	Serum cholesterol	Clinical hypothyroidism	Number. Age in years. Sex	Time after operation	Serum iodine concentration†	BMR	Serum cholesterol	Clinical hypothyroidism
	months	gamma per cent	per cent normal	mgm. per cent			months	gamma per cent	per cent normal	mgm. per cent	
A45443 46 F	2	2.4	+8		0	65313 19 F	7	3.0**	−22	273	1+
	5	6.7	−1		0		9	2.7**	−20	210	2+
95767 13 F	0	3.6	−13		0	A22701 53 F	2	3.8	−4		1+
	1	3.8*	−16		1+		14	2.1	−6		2+
	3	2.3	−13		0	A92514 55 F	0	4.9	+1		0
	4	2.4	−13		0		3	2.0*	+8		0
	5	4.8	−19		0		38	0.5			2+
	6	6.5	−3		0		41	1.4	−10	415	2+
	18	4.7	−7		0	A39971 38 F	2	1.8*	−21	226	1+
	21	4.9	−3		0		3	2.4*	−21	244	2+
B43492 32 F	0	5.0	−2		0		20	2.7	−31		2+
	3	2.5	−21	295	0	B7257 16 F	4	1.3**	−15		1+
	4	5.9	−19	247	1+		6	1.8	−10	300	1+
	6	3.4	−12	254	1+		27	2.0	−10		1+
	7	3.8	−15	262	1+		45	3.8	+4		0
	12	4.1	−2	212	1+		50	3.1*	−1		0
B6138 41 F	5	2.5**	−7	245	0		55	1.8	−14	260	1+
B32017 21 F	1	2.1*	−2	381	1+	A42903 30 F	0	5.6			0
	2	1.6*	−17	376	2+		1	2.7			0
	19	3.2*	−20		0		1	1.5	−4	468	0
	20	3.0	−17		0		3	1.0	−8		0
	21	4.3	−14	325	0		9	1.7	−2		0
10184 42 M	1	2.0**			1+		11	1.1		454	0
	2	2.7**	−2	325	1+	625 40 F	60	2.2**	−14	216	1+
B59454 40 F	2	2.3	−9		0		61	2.5**		178	1+
	5	2.6	−7	195	1+		62	3.8**	−1		1+
	8	1.7	−11	183	2+		63	2.2**	+14	174	1+
B60788 50 F	2	1.5	+7	373	1+		68	2.8	−14		1+
	4	0.0	−5	369	2+		70	4.1	−12		1+
	8	0.4	+3		2+		71	2.2	−11		1+
A35428 63 F	10	3.1	−19		2+		72	3.7	−17		1+
	10	1.3		290	2+	54183 15 F	108	2.8**	−22	453	1+
							144	2.5	−19		1+
							156	2.4	−14	514	1+

TABLE I—*Continued*

Number. Age in years. Sex	Time after operation	Serum iodine concentration†	BMR	Serum cholesterol	Clinical hypothyroidism	Number. Age in years. Sex	Time after operation	Serum iodine concentration†	BMR	Serum cholesterol	Clinical hypothyroidism
	months	*gamma per cent*	*per cent normal*	*mgm. per cent*			*months*	*gamma per cent*	*per cent normal*	*mgm. per cent*	
33751 57 F	2	6.4*	+23		0	A52638 38 F	72	1.3	−27	270	1+
	3	3.2	+49		0						
	5	2.2			0						
B30951 59 F	2	2.1*			0	97827 58 F	300	2.5	+3		1+
	5	3.0*	−30	256	1+		300	3.2		280	1+
							301	2.9	−11	346	1+
B22457 35 M	11	0.6*	−23		2+	A45359 65 F	60	1.5**	−12	319	1+
							63	1.0**	−16	450	1+
A89359 50 F	2	2.3*			2+	B23293 64 F	60	0.6	−36	323	3+
B55943 38 F	1	5.0			0	A48201 60 F	120	1.3	−10	383	3+
	3	3.3			1+						
	5	1.1			2+						
	6		−16		3+						
B37483 36 M	0	4.3	−4		0	35565 42 F	228	1.6	−26	776	3+
	1	2.2	+1		1+						
	6	1.7*	−12		1+						
B69052 45 F	3	2.3		225	1+	B58925 66 F	60	0.7	−25	447	3+
72671 44 F	1	1.6	−18		1+	99644 30 F	72	2.9**	−16	221	0

* Total serum iodine concentration.
** Whole blood iodine divided by 0.6.
† Serum precipitable iodine unless otherwise noted.

ferences in the ability of the remnant of gland left at operation to regenerate properly. This in turn must be conditioned by the radicalism of the operation. The low incidence of recurrent hyperthyroidism and the high incidence of hypothyroidism in the cases whose operations were performed in this hospital during the past 5 years contrasts with the higher incidence of recurrences after operations in this hospital 15 years ago. The difference can readily be explained by the increased radicalism in surgical technique.

It is also quite possible that persistent hypothyroidism was somewhat more common in the past than was recognized at the time. The number of cases in this series of low serum iodine following thyroidectomy performed years earlier is evidence to the point. Before the introduction of the serum iodine technique, diagnosis depended on clinical signs, on the basal metabolism, and on the serum cholesterol. Since postoperative hypothyroidism is, more often than not, partial and clinically equivocal, all of these less sensitive indications may

well fall within normal limits (Table I), thus rendering recognition difficult.

These considerations are all based on the assumption that a low concentration of serum iodine means a low level of output of hormone by the thyroid gland. This in turn is based on the findings in clinical myxedema, in which a low concentration of iodine in serum was invariably found (7), and upon a study of the serum iodine in a series of several hundred subjects without hyperthyroidism in whom a low iodine concentration was never found (9). On the whole, the internal evidence in this study confirms this interpretation of a depression of serum iodine, since most of the subjects had other evidences of thyroid deficiency and responded clinically to thyroid medication. As in spontaneous myxedema, serum iodine rose to normal levels with thyroid therapy in doses ranging from 0.06 to 0.12 grams of dried thyroid daily.

The fall, after iodine medication, of the serum precipitable iodine from a previously elevated level in untreated hyperthyroidism confirms the

observations of others (2, 3). A greater variability was found in response to iodine, both acutely and chronically, than has sometimes been described. Not only may the remission be complete initially but it may sometimes persist for long periods. Iodine medication may even occasionally effect an actual cure. The behavior of the serum precipitable iodine in the 2 subjects of Figure 2 lends support to the theory of Means and others that patients need not become refractory to iodine and that at all times iodine medication exerts some restraining effect on the release of hormone by the hyperthyroid gland.

These observations were not made at sufficiently close intervals to demonstrate clearly a lag in the fall of the basal metabolism behind that of the serum iodine prior to operation, although this was suggested in some individual cases. Postoperatively, the serum iodine often fell to subnormal levels without any further consistent drop in the basal metabolic rate, so that the correlation between the two was poor. The metabolic rate behaved as if, even with sharp decline in serum iodine, an inadequate output of hormone often sufficed to keep it within normal limits.

There are certain clinical implications of these observations. A partial type of hypothyroidism may develop as a sequel to subtotal thyroidectomy, and may often become permanent. It is characterized by mild symptoms of thyroid deficiency and a low serum iodine; basal metabolism is frequently within normal limits and serum cholesterol often only slightly elevated. It may therefore escape detection if these latter measurements are relied on for diagnosis. More severe grades of hypothyroidism may develop, either within a few months or after a delay of some years. Practically, it is obvious from Table I that a basal metabolic rate falling within normal limits is no evidence that thyroid function is adequate.

These findings should not be interpreted as a plea for less radical removal of the gland in subtotal thyroidectomy. The radical operation recommended has fully justified itself in the very low incidence of recurrent hyperthyroidism in this series. Between the Scylla of recurrences and the Charybdis of permanent hypothyroidism, the latter is the lesser evil. The fact that one or the other seems bound to occur in a certain number of cases following subtotal thyroidectomy must, however, be weighed along with the other drawbacks associated with this operation in deciding on the relative merits of operative intervention or more prolonged medical therapy in hyperthyroidism.

CONCLUSIONS

1. The range of values of the serum precipitable iodine in untreated hyperthyroidism is defined. Ninety-five per cent of the values fell above 8 gamma per cent. If the 2 cases who were probably in spontaneous remission are excluded, 98 per cent of the values fell above 8 gamma per cent.

2. The serum precipitable iodine sometimes, but not always, declined with iodine medication in hyperthyroidism, but only attained normal levels in a certain number of cases. Even when given for long periods it continued to exert a depressing effect on the serum precipitable iodine.

3. Subtotal thyroidectomy was followed within 3 hours by a slight rise in serum precipitable iodine, not exceeding a few gamma per cent. Serum precipitable iodine then fell steadily, so that within 3 weeks all supranormal values had disappeared. Later elevations were rare.

4. Subnormal values of the serum iodine might develop within 2 weeks following subtotal thyroidectomy. Although this depression was sometimes transient, these low values in some cases persisted indefinitely.

5. Subnormal values are usually associated with some clinical evidence of mild thyroid deficiency, but the basal metabolism was often within normal limits and the serum cholesterol only slightly elevated. It is probable that low concentrations of precipitable iodine indicate a mild form of partial hypothyroidism. Low serum iodine values are common enough months after subtotal thyroidectomy to indicate that chronic partial hypothyroidism is a not uncommon sequel of subtotal thyroidectomy.

6. The clinical implications of these findings are discussed.

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BALLISTOCARDIOGRAPHIC STUDY OF CHANGES IN CARDIAC OUTPUT DUE TO RESPIRATION [1]

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As a measure of cardiac output the ballistocardiograph leaves much to be desired and the results obtained with this instrument must be accepted with proper caution (Hamilton (1)). Changes in the record can be produced, however, by small variations in experimental conditions which appear to be best interpreted as changes in cardiac output and it seems likely that the method is reliable for relative values when controls are available on the same subject under normal conditions. All the experiments reported in this paper were carried out in this way with a normal record followed immediately by an experimental record on the same subject under altered respiratory conditions; the changes observed are tentatively considered to be due to changes in cardiac output.

The respiratory conditions which we have studied are (*1*) continuous elevated pulmonary pressure, (*2*) intermittently elevated pulmonary pressure, (*3*) voluntary overventilation, (*4*) artificially increased dead space, (*5*) increased alveolar oxygen tension by inhalation of pure oxygen.

METHOD

The ballistocardiograph was designed for us by Dr. H. A. Blair of this laboratory in 1942. The subject reclines on a wooden "bed" made of plywood mounted by means of springs on a massive and rigidly built wooden table. The mountings consist of four straps of spring steel located approximately under each of the four corners of the bed like short legs. These steel straps are 1/16 inch thick and 1 inch wide and 1.75 inches long. They stand vertically and support the weight of the bed. The flat sides face toward the head and foot of the bed so that the bed vibrates longitudinally and the straps bend slightly although imperceptibly. The natural period of vibration of the table is varied by varying the length of the straps. This dimension was so adjusted that the bed made 10 complete vibrations per second with a dead load on the bed of 150 lbs. Movements of the bed were recorded op-

tically by means of a "phoneloscope," made by the Capitol Instrument Co. of Washington, D. C. This is an instrument designed originally to record sound waves and consists of a steel diaphragm, movements of which rotate a small shaft on which a mirror is mounted. Movements of this mirror were recorded optically with an electrocardiographic camera with a magnification of the movement of the diaphragm of about 8000 times. The sensitivity of the apparatus was 0.6 cm. per 100 grams. The diaphragm was connected to the bed of the ballistocardiograph by means of a fine wire under tension of about 100 grams.

In some of our studies we used a vertical ballistocardiograph. This was constructed on the same principle, except that the bed was oriented at an angle of about 15 degrees with the vertical. Arrangements were made so that the subject could either stand on this tilted bed, leaning back against it, or he could sit on a seat which could be attached to the bed when desired. Movements were recorded with a phoneloscope as before. The sensitivity was 0.3 cm. per 100 grams and the frequency was 11 per second. In common with the experience of others, we found the vertical ballistocardiograph much more susceptible to extraneous vibrations and the records much less readable.

Stroke volumes were computed from measurements of the wave heights using two of the formulae of Starr *et al.* (2).

$$SV = K\sqrt{(3I + 2J)A\ C^{3/2}}$$

and

$$SV = K\sqrt{J\ A\ C^{3/2}}.$$

The latter formula was used only in the first series of measurements on the horizontal apparatus.

In these equations K = constant for the apparatus; I = height in mm. of the I wave (first downward deflection); J = height in mm. of the J wave (subsequent upward

FIG. 1. SAMPLE RECORD DURING NORMAL BREATHING
Time in half seconds

413
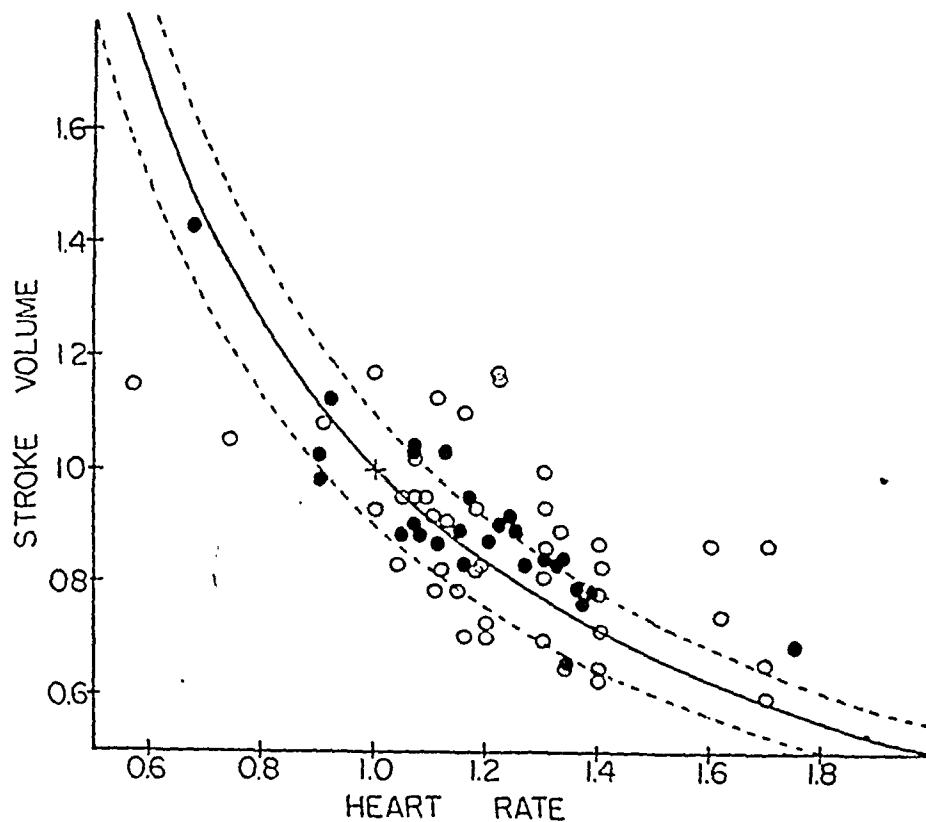
FIG. 2. THE EFFECT OF BREATHING AGAINST POSITIVE PRESSURE ON THE
STROKE VOLUME, AND HEART RATE

Each point represents a comparison between normal and experimental conditions, heart rate and stroke volume being expressed each as a fraction of the normal value taken as 1.0. The normal is therefore represented by the cross (+) at point 1.0, 1.0 on the diagram, and all the points are relative experimental values. The solid line is a rectangular hyperbola passing through the cross and indicating points where the product of heart rate and stroke volume, or the cardiac output, is constant and normal. Broken lines represent cardiac output 10 per cent above (on the right) or below (on the left) normal. Open circles = horizontal, and closed circles = vertical ballistocardiograph. Note that the points lie in general to the right of the cross (increased heart rate) but below (decreased stroke) so that they scatter more or less uniformly both above and below the solid curve. This indicates no average change in cardiac output.

deflection); A = aortic cross section in sq. cm.; C = duration in seconds of one cardiac cycle.

In most cases we made no effort to calculate the cardiac output or stroke volume in absolute units, but determined only relative magnitudes.

A typical normal record is shown in Figure 1, demonstrating that the tracings are clear and easily measured and in general similar to those published by Starr *et al.* (2).

RESULTS

(1) Changes in intrapulmonary pressure

In this series of experiments positive pressure was applied to the lungs, either through a mouth piece for short periods or for longer periods through a

helmet which covered the whole head and which was sealed around the neck with a Drinker respirator type of sponge rubber collar. The subject breathed air of which the pressure was controlled between 25 and 30 cm. H_2O by a limit valve, and the flow was sufficient to prevent the accumulation of carbon dioxide. The mouthpiece could not be used for long periods on account of the resulting fatigue of the cheek muscles. In the sitting position we have 14 experiments on 8 different male subjects; in the supine position, 17 experiments on 14 subjects. The results may conveniently be summarized in Figure 2, in which the ordinates represent stroke volumes as a fraction of the nor-

mal stroke volume, and abscissae represent heart rates expressed as a fraction of the normal heart rate. In such a graph all points with a normal cardiac output of 1.0 should lie along a rectangular hyperbola passing through the point 1, 1, marked by a cross. The dotted lines indicate cardiac outputs 10 per cent greater or less than this normal. Values in the sitting position are indicated by solid circles and those in the supine position by open circles. It is evident from this chart that in most cases there is little change in cardiac output, although some subjects show an increase and some a decrease. However, there is a well marked tendency for the stroke volume to decrease and the heart rate to increase enough to compensate. In a few cases the heart rate decreased and the stroke volume increased. The two points showing the greatest decrease in cardiac output are on the same subject, a 19-year-old girl whose heart failed to accelerate enough to maintain the cardiac output even though the stroke volume was above normal.

The grand average of all the figures shows an increase of heart rate of 1.18 times while the stroke volume decreased to 0.89 of the normal. The product of these two gives a cardiac output 1.05 times the normal. From the same experiments average values, including both sitting and supine figures, have also been obtained at different times from 15 seconds to 10 minutes after the beginning of pressure breathing, but no consistent change with time could be discerned. The heart rapidly adjusts itself to the unusual conditions and maintains this adjustment with a fairly constant value. On the average, the cardiac output, so measured, does not differ significantly from the normal at pressures up to 30 cm. H_2O, although there are certain individuals at certain times who do show a decrease.

Higher pressures for short periods of time have been tested in two subjects who were asked to exert voluntary pressure against a mercury manometer as in the Flack test for circulatory efficiency (3) or the test outlined by Bürger (4). In these cases the stroke volume did diminish, but the heart rate increased enough to more than compensate so that in one subject at 50 mm. Hg. the cardiac output was 158 per cent of normal, while in the other at 40 mm. Hg. it was 119 per cent of normal.

For purposes of comparison, a few experiments

have been carried out on subjects breathing under conditions of negative pulmonary pressures of − 30 cm. H_2O. Under these conditions also no significant change in cardiac output was found. For 12 cases for periods up to 4 minutes the average values relative to the normal were: stroke volume, 1.0, heart rate, 1.1, cardiac output 1.1.

It should be pointed out that all these experiments were carried out on untrained subjects, largely medical students. The application of positive pulmonary pressure by mouthpiece becomes quite uncomfortable in a very short time and application of pressure via a helmet is no more reassuring to the uninitiated subject. The average time at which these records were taken is less than 3 minutes and one-third of the records were taken less than a minute after applying pressure. In view of the necessary anxiety and short period for adjustment the quantitative results must be accepted with some reservations.

Consequently, another series was obtained on 6 subjects who had had previous experience with pressure breathing in connection with other experiments. Ample time was allowed for these men to adjust themselves to the positive pressure which was administered through a mouthpiece, mask or helmet, and records were taken 10 to 40 minutes later. The results are shown in Table I, all values being expressed in percentage of normal. The results were consistent in showing an increase in heart rate, decrease in stroke volume, a decrease in cardiac output and pulse pressure

TABLE I

Effect of positive pulmonary pressure of 30 cm. H_2O

Sub-ject	Heart rate	Stroke vol.	Cardiac output	Systolic pressure	Diastolic pressure	Pulse pressure
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
RD	115	71	82	107	113	98
PB	113	76	85	108	120	84
WF	111	86	98	104	109	96
PS	115	65	75	100	110	85
AO	111	83	94			
AC*	122	65	79	106	115	91
Average	115	74	86	105	113	91

* These results are averages of many experiments on a single subject with 30 cm. H_2O applied in different ways as follows: 7 experiments using an aviation mask, 6 experiments using a helmet, 4 experiments with a mask and a pneumatic vest (Mae West) for the application of counter pressure to the chest, 12 experiments with a helmet and vest and 1 experiment using a mouth piece.

All quantities in percentage of normal.

TABLE II

*Effect of artificially forced respiration (Pneumolator)
on the cardiac output.*

Subject	Heart rate	Stroke volume	Cardiac output
	per cent	*per cent*	*per cent*
RD	89	102	90
HR	112	77	86
AH	93	102	95
AO	78	93	72
Av. of 6*	96	90	85
Grand Av.	95	91	85

* Individual data on these 6 subjects are presented in Table III. The peak pressure on the pneumolator was 20 to 25 cm. H_2O, frequency 7 to 9 per minute.

All values in percentage of normal.

but an increase in both systolic and diastolic pressure.

The last line in Table I summarizes a number of experiments on one subject (A.C.) who breathed against 30 cm. H_2O positive pressure applied with a mask or with a helmet, and with and without counter-pressure from a pneumatic vest. Only average figures of all the experiments are given because the variations in the method of application of the pressure made no difference. All the experiments were similar qualitatively and showed on the average a 14 per cent decrease of the cardiac output. This figure approaches the results of Richards (5) obtained by the direct Fick method on 4 patients breathing 27 cm. H_2O.

(2) Effects of intermittently elevated pressure with the pneumolator

Some further experiments were tried with intermittent pressure breathing or artificially forced respiration. For this purpose we used an apparatus built by the General Electric X-ray Corporation called a pneumolator. This instrument supplies oxygen or air to a mask which is inflated along with the lungs until a critical pressure is reached. At this point the inflow of oxygen automatically stops and an expiratory port is opened so that the pressure can slowly fall to zero at a rate which can be controlled by a variable gate. The value of the peak pressure can be controlled for each experiment. As soon as the pressure reaches zero the expiratory port is closed and inflow starts again. The mean pressure in the lungs during a cycle is about 40 per cent of the peak

pressure, so this is a form of pressure breathing. Since, however, the pressure is higher in inspiration than in expiration, the process tends to increase the depth of respiration and lowers the alveolar carbon dioxide tension. Breathing through this apparatus is easier than against a continuous pressure, but it is insidious because it is likely to lead to acapnia.

The results obtained with this type of breathing are shown in Table II. The average stroke volume is 91 per cent of normal with a mean pulmonary pressure of 8 to 10 cm. H_2O. With continuous pressure breathing the stroke volume was decreased to 74 per cent of normal with a mean pulmonary pressure of 30 cm. H_2O. It may be concluded, therefore, that the stroke volume is decreased in both cases in proportion to the mean pressure.

The data of Table II (and Table III) show that 6 out of 10 subjects breathing with the pneumolator had decreased heart rates, the average being 95 per cent of normal. This result contrasts sharply with the figures in Table I where all the subjects uniformly showed increased rates of 11 to 22 per cent over the normal. Largely due to this difference in the reflex response of the heart, the use of the pneumolator results uniformly in a decreased cardiac output averaging 85 per cent of normal.

The reason for the difference in heart rate with the two kinds of pressure breathing is not obvious, but it might possibly be attributed to the greater muscular effort involved when the pressure continues during the expiratory phase.

Brief mention should be made of some other experiments which were undertaken with the idea that the effects of pressure breathing might be artefacts due merely to the change in the degree of inflation of the chest. With a larger chest it was thought that the impacts from the heart might be less well transmitted to the body as a whole. In these experiments the subject held his breath with the glottis open at different degrees of inspiration and expiration while records were taken on the ballistocardiograph. The results showed in general a decreased heart rate at the higher degrees of inspiration in agreement with the findings of Lewis, Lewis, and Hall (6), and *vice versa* for expiration, but no significant change in the stroke volume. It is perhaps significant that the stroke

TABLE III

Effect on cardiac output of pneumolator (Pneu) and voluntary hyperventilation (VHV) at the same alveolar pCO_2

Subject	Heart rate			Stroke volume		Cardiac output		Alveolar pCO_2		
	Normal	Pneu	VHV	Pneu	VHV	Pneu	VHV	Normal	Pneu	VHV
	beats per min.	per cent	per cent	per cent	per cent	per cent	per cent	mm. Hg.	mm. Hg.	mm. Hg.
M.E.	73	111	114	89	87	99	99	40.6	28.2	23.4
M.H.	67	84	93	106	107	88	99	38.7	25.0	25.1
M.B.	62	110	108	93	96	103	105	38.2	27.4	22.8
J.H. (a) (b)	64	98 100	108	76 74	86	74 73	92	46.3	25.7 24.9	
H.R. (a) (b)	78	97 90	100	83 96	93	81 85	93	42.9	28.7 28.5	30.0
B	72	88 89	81	98 93	115	84 82	92	36.5	32.7 34.0	29.8
Average	69	96	101	90	97	85	97	40.6	28.4	26.2

The peak pulmonary pressure on the pneumolator was 20 to 25 cm. H_2O. All values in percentage of normal unless otherwise indicated.

volume did not increase in the inflated position in spite of the decreased heart rate. The changes in the heart rate indicate some reflex cardiac effects resulting from different degrees of inflation. This does not help to explain the effects of continuous and intermittent pressure on the heart rate, because the mean lung volume was greater with the former where the heart rate was highest.

(3) Effect of decreased alveolar carbon dioxide tension

It has been shown that for the same mean pulmonary pressure the cardiac output is more diminished by intermittent than by continuous pressure breathing. This suggests the possibility that the diminished alveolar carbon dioxide tension which accompanies the use of the pneumolator may be responsible for this difference. In order to investigate this hypothesis one of us (M.B.) carried out a series of ballistocardiographic determinations on 6 different subjects in which the effects of voluntary hyperventilation were compared to the effects of breathing on the pneumolator.

These experiments involved three periods: (1) 10 minutes reclining in the supine position on the ballistocardiograph breathing air normally through an A-13 mask; (2) 10 minutes breathing air through the pneumolator with a peak pressure of

20 to 25 cm. H_2O; (3) 10 minutes of voluntary hyperventilation. At the close of each period a record was taken on the ballistocardiograph followed by an alveolar air sample taken from a side tube in the mask after a forcible expiration (beginning at the end of expiration).

The results of these measurements are shown in Table III. They indicate that the cardiac output was decreased to 85 per cent of normal by the pneumolator but to only 97 per cent of normal by voluntary hyperventilation. The fall in the alveolar carbon dioxide tension was approximately the same in both cases. Similar results were obtained by Drs. Kety, Schmidt, and Starr, University of Pennsylvania (Personal Communication). The experiments with the pneumolator confirm those reported in Table II. In a second series of experiments air was delivered to the mask through a gas meter which stood in view of the subject. Every 5 seconds the subject inhaled and continued until the pointer had reached a certain point on the dial. In this way the volume of the ventilation could easily be controlled either at 12 or 18 liters per minute. The experiment consisted then of a 10-minute normal period with the mask but without the gas meter, then two 6-minute periods with the gas meter at each of the two increased tidal volumes. Records and alveolar air samples were taken near the end of each period. Three

experiments were tried on each of 5 subjects. In order to avoid disturbance of the ballistocardiographic record by the mechanical oscillations due to the increased effort of breathing, the subject was instructed to breathe normally while the record was being taken. All records were taken within 30 seconds after the cessation of hyperventilation so that it can be fairly assumed that any effects of lowered alveolar carbon dioxide tension would still be in operation.

The results of these experiments are summarized in Table IV. All the changes are very small

TABLE IV

Effects of voluntary hyperventilation of different degrees on cardiac output. Averages of 3 experiments on each of 5 subjects

Ventilation	Heart rate	Stroke volume	Cardiac output	Alveolar pCO$_2$
liters per min.	*per cent*	*per cent*	*per cent*	*mm. Hg*
Normal	100	100	100	44.1
12	103	96	99	34.0
18	103	92	95	24.0

but there is a well marked tendency toward a decreased cardiac output (95 per cent) and a decreased stroke volume (92 per cent) which was not balanced by an increase in heart rate (103 per cent) at the higher rate of ventilation. Intermediate values were obtained at the 12 liter per minute rate. The alveolar CO$_2$ tension at the higher rate was decreased to 24 mm. Hg which is comparable to the decrease observed in Table III, and the decrease in cardiac output is of the same order of magnitude. The uniformity of the 15 experiments was shown by the fact that at 18 liters per minute 11 experiments showed a decrease in cardiac output, 13 showed a decrease in stroke volume and 13 showed an increase in heart rate or no change. The 5 per cent decrease in cardiac output was statistically significant because the probable error of the mean was only ± 1.1 (Table I

In considering the significance of these re it might be pointed out that increased venti requires increased oxygen consumption. Ad ing that the cardiac output is increased in at a tion to the oxygen consumption, it is p gate. predict on the basis of figures for the o trolled sumption in overventilation given by essure (7), that the cardiac output will be nd in per cent at 12 liters per minute and 7 e lungs le peak

18 liters per minute. From these figures it might be argued that if the same ventilation could have been produced passively, so as to avoid the increased cardiac output required for this increased metabolism, the cardiac output would have been only $99 - 3 = 96$ per cent at 12 liters per minute, and $95 - 7 = 88$ per cent of normal at 18 liters per minute. Such figures actually were closely paralleled by those obtained with the pneumolator where the ventilation was passive although the pressure was elevated.

These results appear to indicate that there is a small but distinct decrease in cardiac output due to diminished alveolar pCO$_2$ but the situation is of course very complicated and the result might be attributed equally well to mechanical effects of the altered respiration. This result also does not permit the conclusion that the decrease in cardiac output observed with the pneumolator is due to the hypocapnia which is produced, because the high pulmonary pressure provides an equally good explanation. In addition, it might be mentioned that Grollman (8) using the acetylene method for cardiac output found an increase with forced ventilation, even when a fall of alveolar pCO$_2$ was prevented by the inspiration of suitable CO$_2$ mixtures. The reason for this discrepancy is not clear.

(4) Experiments with increased dead space

To complete this study some further measurements were made of the effects of increased alveolar carbon dioxide tensions obtained by increasing the dead space. The subject breathed air through a mask and 3-way cock. The two free ends of the cock were connected to open tubes containing 210 and 760 ml. respectively. By turning the cock the subject could be made to breathe in and out through either one of these tubes. Normal records were taken without the mask. Each period was continued for 15 minutes before the record was taken. This experiment was tried on each of 5 subjects. The results were variable and inconsistent. On the average, the addition of either of the dead spaces increased the cardiac output over the normal value, but this difference was certainly not significant. Other experiments have shown that the alveolar pCO$_2$ was increased not more than 0.5 and 5 mm. Hg by the 210 and 760 ml. respectively.

This experiment indicates that an increase of alveolar CO_2 does not increase the cardiac output, and this is consistent with the conclusions reached by Grollman (8) on the basis of the acetylene method. Indeed, if allowance is made for the increased metabolic rate which must have accompanied the increased breathing needed for these dead spaces (about 18 liters per minute with 760 ml.) the effect of the alveolar change by itself might well have been a decrease in the cardiac output.

(5) Effects of oxygen inhalation

Before considering the ballistocardiographic data, mention should be made of a series of experiments in which the minute volume of the ventilation was measured in three successive periods on air, then pure oxygen, and finally on air. Our purpose was to investigate some of the rather large increases of ventilation produced by oxygen breathing which had been reported, averaging 20 per cent according to Edelmann, Whitehorn, and Hitchcock (9) and 13.4 per cent according to Shock and Soley (10). All our subjects came early in the morning without breakfast and reclined for 30 minutes on a bed before the experiment began. Air or oxygen was inhaled from a spirometer through a mouth piece and valves. Each period lasted for 30 minutes and was followed by a 10-minute rest period. Ventilation was determined from the spirometer which had a capacity of 2 liters per cm. with a total of about 100 liters. In some of the experiments it was the expired air which was collected in a spirometer and measured. Results were corrected to body temperature, pressure and saturation (BTPS). Table V gives the results of 16 experiments on 6 different subjects. In the last two columns the ventilation on oxygen is expressed in percentage of the two control ventilations on air. On the average it is seen that oxygen increases the ventilation 5 ± 1.2 per cent compared to the first control, and 6 ± 0.89 per cent compared to the second control period. The standard deviations of the mean indicate a very high degree of statistical validity. It is concluded that oxygen increases the minute volume of the ventilation only 5 or 6 per cent, few if any of our figures being as high as those reported previously.

In 10 of the experiments (5 subjects) listed in Table V simultaneous measurements were made

TABLE V

The volume of the ventilation in liters per minute breathing oxygen and air

Subject	Air (1)	O₂	Air (2)	Ventilation in O₂ in percentage of air	
	liters per min.	liters per min.	liters per min.	(1)	(2)
HR	6.89	7.72	7.54	112	102
HR	7.64	8.35	7.50	109	111
HR	6.08	6.40		105	
J.H.	6.74	7.15	6.45	106	111
J.H.	6.40	6.54	6.50	102	101
H.M.	5.46	5.54	5.28	101	105
H.M.	5.48	5.76	6.11	105	94
H.M.	5.60	6.18	5.81	110	106
A.C.	6.86	7.27	7.14	·106	102
A.C.	8.05	7.66	7.06	95	108
A.C.	7.85	7.99	7.20	102	111
A.C.	6.29	6.83	6.77	108	101
H	6.16	7.05	6.32	114	111
H	7.09	7.50	6.67	106	112
H	6.76	7.00	7.08	104	99
J.H.	5.35	5.43	4.77	101	114
Average	6.55	6.90	6.53	105	106
St. dev.	±.85	±.87	±.79	±4.7	±3.4
St. dev. of mean.	±.21	±.22	±.20	±1.2	± .8°

of the stroke volume and heart rate with the ballistocardiograph. The results are summarized in Table VI. As before, the values during the oxygen period are calculated in percentage of the two control periods. The fact that these two sets of control values agree so closely shows that no progressive change was occurring in the condition of the subject. The figures show a decrease in the cardiac output during oxygen breathing in every case, averaging 8.5 per cent with a standard deviation of the mean of 1 per cent. Since the stroke volume showed on the average no change, it must be concluded that the decrease in the heart rate was responsible for the decrease in cardiac output. These results agree with those reported by Whitehorn, Edelman, and Hitchcock (11), except that they found a slightly larger decrease in cardiac output amounting to 12 per cent and found the stroke volume decreased as well as the heart rate.

DISCUSSION

The main question relative to these experiments is whether our procedure has actually measured the cardiac output. In this respect it is satisfying to

TABLE VI

Effects of oxygen inhalation on cardiac output, heart rate and stroke volume

Subject	Compared to first control period			Compared to second control period		
	Heart rate	Stroke volume	Cardiac output	Heart rate	Stroke volume	Cardiac output
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
H.R.	83	106	88	96	97	93
H.R.	83	108	90	93	100	94
J.H.	95	101	94	95	102	96
J.H.	90	98	87	96	94	91
T.H.	87	104	90	94	99	93
T.H.	93	96	89	90	110	96
A.C.	94	97	92	83	105	87
A.C.	100	92	92	84	101	85
H.M.	92	100	93	91	101	92
H.M.	97	96	93	99	97	95
Mean	91.4	99.8	90.8	92.1	100.6	.92.2
Stand. dev.	5.6	5.3	2.4	5.2	4.5	3.7
Std. dev. of mean	1.8	1.7	0.7	1.7	1.4	1.2

Values in percentage of control periods on air taken before ___after the oxygen period.

but that in our trained subjects using continuous pressure breathing, and in all our subjects using intermittent pressure breathing, the cardiac output appeared to be reduced in proportion to the mean pulmonary pressure. This is in agreement with the results reported by Richards (5), with the direct Fick method. We have no similar confirmation of our results with forced breathing where a slight decrease in cardiac output also was found. This is contradictory to the results reported by Grollman (8) with the acetylene method, but may nevertheless be correct for our experimental conditions. There are so many conflicting chemical and mechanical factors involved in forced breathing that either result might be expected according to the exact pattern of the breathing.

The respiratory and circulatory effects of substituting pure oxygen for air are more uniform and indicate a 5 per cent increase in the minute volume of the ventilation and an 8 per cent decrease in the heart rate and cardiac output. Speculation concerning the mechanism of these effects seems futile because of the many possibilities which present themselves. However, it might be pointed out that the initial stimulus presumably depends upon the increased pO_2 in the arterial blood. Because of the decreased cardiac output, as well as the increased rate of oxygen consumption reported by Behnke, *et al.* (12), the mean pO_2 in the tissues would not be expected to change very much. The result, however, may depend more upon the response of the cerebral circulation to pure oxygen, and this may be quite different from the average for the whole body.

SUMMARY

(1) Records were taken with a ballistocardiograph to show the effects on the circulation of variations in intrapulmonary pressure or alveolar carbon dioxide tension, and of pure oxygen inhalation in place of air. The results are interpreted tentatively in terms of cardiac output.

(2) Breathing against a continuously elevated pulmonary pressure decreases the stroke volume but increases the heart rate. Compensation may or may not be complete.

(3) Breathing against a pulmonary pressure which is elevated only during inspiration decreases the stroke volume, in proportion to the mean pulmonary pressure, as well as the heart rate, so that the cardiac output is decreased to 85 per cent of normal.

(4) In voluntary hyperventilation of approximately the same volume as in *(3)* the cardiac output is diminished to 95 per cent of normal, the stroke volume being decreased and the heart rate increased.

(5) An increase in the dead space caused no consistent effect on cardiac output.

(6) Substitution of pure oxygen for air in normal breathing resulted in an increase of 5 per cent in the volume of the ventilation and a decrease of 8.5 per cent in the heart rate and cardiac output.

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PROPERTIES OF AN ANTICOAGULANT FOUND IN THE BLOOD OF A HEMOPHILIAC

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Munro and Jones (1) have reported on a case of hemophilia who, apparently as the result of frequent transfusions, had developed a phase in which further transfusions failed to cause either a reduction in his coagulation time or clinical improvement. At that time it was demonstrated that the blood of this patient contained an anticoagulant. It was suggested that the numerous transfusions were responsible for the development of this anticoagulant. Further study has tended to confirm this interpretation.

There have been two previous reports of the presence of an abnormal circulating anticoagulant. Lawrence and Johnson (2) have presented a study on a hemophiliac similar to this case. The diagnosis of hemophilia was established by both the clinical findings and the family history. The patient, however, was refractory to transfusions and had a circulating anticoagulant with properties closely allied to those found in this case. They reported that he had received many transfusions but did not state whether the anticoagulant was known to be present during a period when transfusions were not given. Lozner, Jolliffe and Taylor (3) have described a 61-year-old Negro who had a circulating anticoagulant which they were unable to identify. In this case the diagnosis of hemophilia was excluded on the basis of both the past history and the family history.

Tocantins (4) has described a normally occurring anticoagulant which he names antithromboplastin, or anticephalin. He has found this substance to be abnormally high in hemophilia and considers it to be the cause of the prolonged coagulation time in this disease. As will be shown, the anticoagulant discussed here is not anticephalin.

It is the purpose of this paper to describe some of the properties of this anticoagulant. Further clinical observations on this patient will be discussed in a later paper.

EXPERIMENTAL PROCEDURE

Studies with this anticoagulant in unfractionated plasma showed that it could most easily be studied by the following technique: blood was collected from the patient by venepuncture and mixed with ⅑ of its volume of 0.1 M sodium oxalate. It was then centrifuged for 1 hour at 2000 rpm and the plasma removed. Various quantities of the plasma were then added to 0.4 ml. of normal plasma prepared in the same manner. The mixture was recalcified by the addition of an equal volume of 0.025 M calcium chloride, the total volume being adjusted to 1.2 ml. by the addition of the requisite amount of 0.15 M sodium chloride. A typical experiment showing the effect of increasing amounts of this plasma is given in Table I. Experience showed that 0.2 ml. of this plasma with 0.4 ml. of normal plasma generally gave a satisfactory coagulation time. Accordingly, in studying the various properties of the anticoagulant the coagulation time was determined in a mixture containing 0.2 ml. of this plasma, 0.4 ml. of normal plasma, and 0.6 ml. of 0.025 M calcium chloride. Since this procedure was followed in a majority of the experiments the details will not be repeated for each one. Variations from it will be described in any particular case.

RESULTS

The studies made on this anticoagulant may be divided into three main groups. These are: (*1*) the properties of the anticoagulant in unfractionated plasma, (*2*) the relation of the anticoagulant to the various factors involved in the coagulation mechanism, and (*3*) the fractionation of the plasma with the object of determining the nature of the active material.

TABLE I

The anticoagulant action of the patient's plasma on normal plasma

Patient's plasma	Normal plasma	Sodium chloride 0.15 M	Calcium chloride 0.025 M	Coagulation time
ml.	*ml.*	*ml.*	*ml.*	*min.*
0.00	0.40	0.40	0.40	3
0.05	0.40	0.30	0.45	4
0.10	0.40	0.20	0.50	12
0.15	0.40	0.10	0.55	43
0.20	0.40	0.00	0.60	50
0.20	0.00	0.40	0.20	150

(1) Properties of the anticoagulant in unfractionated plasma

The effect of incubating the patient's plasma with normal plasma

The patient's plasma was mixed with twice its volume of normal plasma and incubated at 37° C. At intervals, as shown in Table II, 0.6 ml. of the mixture was removed, 0.6 ml. of 0.025 M calcium chloride added, and the clotting time determined. At the same intervals a fresh mixture containing 0.2 ml. of the patient's plasma and 0.4 ml. of normal plasma was prepared and recalcified in the same way. The clotting time of the incubated mixture and the newly prepared mixtures were parallel over a period of 24 hours. It appears, therefore, that the activity of this anticoagulant is not decreased by incubation with normal plasma.

The effect of storage and temperature

Plasma prepared in the usual way was tested for its anticoagulant activity on the day of collection and stored in the refrigerator. At intervals the plasma was tested for its anticoagulant activity against fresh normal plasma. As shown in Table III, no decrease in anticoagulant occurred during a period of 10 days.

Quantities of 5 ml. of the plasma were heated for 5 or 10 minutes at various temperatures. The samples were then centrifuged to remove any precipitate which had formed and tested for anticoagulant activity. There was no decrease in the activity in any of the samples (Table IV).

TABLE II

The effect of incubating the patient's plasma with normal plasma

Incubation time	Coagulation time*	
	Incubated	Not incubated
hours	*min.*	*min.*
0		20
1	22	18
3	18	14
4	23	19
5	27	27
24	22	25

* Each test was made using 0.2 ml. of the plasma, 0.4 ml. of normal plasma, and 0.6 ml. of 0.025 M calcium chloride.
Coagulation time of normal plasma 6 min.

TABLE III

The effect of storage

Storage time days	Coagulation time* min.
0	42
1	40
2	40
4	41
10	46

* Each test was made using 0.2 ml. of the plasma, 0.4 ml. of normal plasma and 0.6 ml. of 0.025 M calcium chloride.
Coagulation time of normal plasma 6 min.

TABLE IV

The effect of temperature

Temperature	Time	Coagulation time*
°C.	min.	min.
		22
45	5	24
55	5	24
65	5	27
65	10	24

* Each test was made using 0.2 ml. of the plasma, 0.4 ml. of normal plasma, and 0.6 ml. of 0.025 M calcium chloride.
Coagulation time of normal plasma 6 min.

These data indicate that the anticoagulant is very stable to both storage and heat. The only normally occurring anticoagulant which is not inactivated by the amount of heat applied to this plasma is antithrombin (5). As will be shown later, it has not been possible to demonstrate an increase in antithrombin in this plasma. Tocantins (4) has studied the effect of heating on anticephalin. He finds that anticephalin is not stable at temperatures above 50° C. It appears, therefore, that this anticoagulant is not anticephalin.

The relative activity of oxalated, citrated or unmodified whole blood and plasma

Blood from the patient was handled as follows: one part was mixed with 1/9 of its volume of 0.1 M sodium oxalate, a second with 1/9 of its volume of 3 per cent sodium citrate, and the remainder left without anticoagulant. Studies were made of the anticoagulant activity of the three blood samples and the three corresponding plasma samples. This was done by adding the necessary amount of calcium chloride and then adding 1.0 ml. of freshly drawn unmodified normal blood. Tests on all six materials were performed at the same time, approximately 90 minutes after collecting the blood.

TABLE V

The relative activity of oxalated, citrated or unmodified whole blood and plasma

Anticoagulant	Coagulation time of*	
	Whole blood	Plasma
	min.	*min.*
None	20	45
Sodium Oxalate	20	35
Sodium Citrate	20	36

* 1 ml. of blood, or 0.55 ml. of plasma were mixed with 1 ml. of normal blood.

Coagulation time of normal blood 6 min.

All the preparations of both blood and plasma had a definite anticoagulant effect (Table V). The plasma samples containing oxalate or citrate when recalcified showed less activity than the unmodified sample. This decrease in activity is probably due to lysis of the platelets which occurs during the process of decalcification and subsequent recalcification. This process, resulting in an increased supply of thromboplastin, would tend to accelerate the clotting mechanism. In the whole blood samples there was no difference between the samples containing oxalate or citrate and the unmodified sample. In this case the number of platelets was not decreased by centrifuging and the continued disintegration during the period of standing would result in the decreased clotting time observed with the whole blood as compared to the plasma.

The effect of hydrogen ion concentration

Quantities of 3 ml. of the plasma were adjusted to various hydrogen ion concentrations from pH 5.0 to 11.0 and left at these concentrations for 3 hours. They were then returned to pH 7.6 to 7.8 and tested for anticoagulant activity. Precipitation occurred in the samples brought to pH 6.0 and below and pH 10.0 and above. On neutralizing, these precipitates redissolved in the pH 6.0 and 10.0 samples, but the others remained turbid. The anticoagulant activity decreased markedly in the samples brought to pH 5.0 and 5.5 and slightly in the sample brought to pH 6.0. There was no alteration in activity in any sample from pH 6.5 to 11.0 inclusive (Table VI).

(2) *Relation of the anticoagulant to various components of the coagulation mechanism*

Quick (6) in his discussion of anticoagulants lists five possible types, namely decalcifying agents,

antiprothrombins, antithromboplastins, fibrinogen antagonists, and antithrombins. Studies have been made of this anticoagulant with the object of determining which component of the coagulation mechanism it affected. Since it has so far not been possible to demonstrate that this anticoagulant falls into any of the above classes, the experiments made to determine its type will be described only briefly.

The concentration of calcium chloride used in recalcifying this plasma alone, and mixtures of this plasma with normal plasma was varied over a range of 0.015 M to 0.050 M. The minimum coagulation time in each case was obtained at the same concentration as that for normal plasma, namely 0.025 M calcium chloride.

The prothrombin time of the plasma has been determined by the one-stage method on various occasions. It has been found to be the same as that of normal plasma, which would not be the case if an antiprothrombin were present.

While plasma from this patient has been shown to contain antithromboplastin by the special methods described by Tocantins, the procedures by which it has been handled in this laboratory are such that antithromboplastin would become inactivated. Blood has been collected without special precautions and treated in numerous ways which are reported to inactivate antithromboplastin without any resulting inactivation of this anticoagulant. For these reasons as well as the differences in stability and chemical behavior described elsewhere in this paper, the data indicate that if this anticoagulant is an antithromboplastin, it is not the same antithromboplastin that has been described by Tocantins.

While the coagulation of this plasma, either

TABLE VI

The effect of hydrogen ion concentration

pH	Coagulation time*
	min.
5.0	29
5.5	34
6.0	39
6.5	45
8.0	46
9.0	50
9.5	45
10.0	53
11.0	45

* Each test was made using 0.2 ml. of the plasma, 0.4 ml. normal plasma and 0.6 ml. 0.025 M calcium chloride.

Coagulation time of normal plasma 3 min.

alone or mixed with normal plasma, was delayed, the clot which formed was normal. For this reason it was not considered probable that the anticoagulant was a fibrinogen antagonist.

Determination of the antithrombin activity of this plasma by the method described by Wilson (7) showed that there was no increase in antithrombin compared to that of normal plasma. Addition of protamine [1] in quantities of 0.001 to 0.1 mgm. per ml. of plasma or whole blood caused no reduction of the clotting time. Since protamine is known to be a precipitant of heparin it may be assumed that the anticoagulant is not heparin.

The prothrombin conversion time of the plasma determined by the two-stage method of Warner, Brinkhous and Smith (8) was found to be the same as that of normal plasma. This observation also confirms the view that the anticoagulant is not an antithrombin, since antithrombin is known to prolong the prothrombin conversion time.

(3) Fractionation of the plasma

Separation of albumin and globulin

Albumin and globulin were prepared from the plasma by precipitation with 21 per cent sodium sulfite (9). In one experiment the globulin was precipitated by direct addition of 19 volumes of 21 per cent sodium sulfite to the plasma. The precipitated globulin was centrifuged down, washed once with 21 per cent sodium sulfite and recentrifuged. It was then dissolved in 0.15 M sodium chloride and dialyzed against 0.15 M sodium chloride until free of sulfite.

Since this procedure diluted the albumin to such an extent that activity would not be demonstrable if present, a second precipitation was performed by dialysis of 10 ml. of the plasma against 190 ml. of 21 per cent sodium sulfite until equilibrium was reached. The contents of the dialysis bag were then centrifuged, the supernatant containing the albumin removed, the precipitate dissolved in 0.15 M sodium chloride and both fractions were dialyzed against 0.15 M sodium chloride until free of sulfite.

The results of this experiment (Table VII) in-

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[1] We are indebted to Dr. Irvine H. Page of the Lilly Research Laboratories, Indianapolis, Ind., for the protamine used.

TABLE VII

The activity of albumin and globulin fractions

Fraction	Coagulation time*
	min.
Whole plasma	44
Globulin prepared by direct precipitation	43
Globulin prepared by dialysis	60
Albumin prepared by dialysis	4

* Each test was made using 0.2 ml. of the plasma or fraction, 0.4 ml. normal plasma and 0.6 ml. of 0.025 M calcium chloride.

Coagulation time of normal plasma 4 min.

dicate that the anticoagulant activity is precipitated with the globulin. Both globulin preparations had an activity equal to that of the plasma from which they were derived, while the albumin had no anticoagulant effect.

Adsorption with aluminum hydroxide gel

Aluminum hydroxide gel has been used extensively as an adsorbent of various proteins. In the field of blood coagulation it has been used in the preparation of prothrombin-free plasma (10). It appeared of interest to determine whether alumina gel would affect the activity of this anticoagulant.

The plasma was mixed with 1/10 of its volume of alumina gel and incubated at 37° C. with frequent stirring for 20 minutes, the alumina gel removed by centrifuging and the procedure repeated. This plasma had a prothrombin time by the one-stage method of over 3 minutes. There was no change in the anticoagulant activity of the preparation compared to that of untreated plasma. So the anticoagulant is not adsorbed by alumina.

Euglobulin preparation

Considerable stress has been placed on the rôle of euglobulin preparations, of various types, in the field of blood coagulation. This fraction, prepared by dilution and isoelectric precipitation, is the starting material for the preparation of prothrombin (11). Euglobulin from normal plasma prepared either by dilution and isoelectric precipitation or by dialysis has been shown to contain a substance which accelerates the clotting of hemophilic blood, both *in vivo* and *in vitro* (12). A similar preparation has been shown to contain anticephalin (4). From this very incomplete discussion of the properties of euglobulin it is apparent

TABLE VIII

Activity of euglobulin prepared by isoelectric precipitation

Fraction	Coagulation time*
	min.
Untreated plasma	16
Euglobulin	1
Alumina-treated plasma	15
Euglobulin	3

* Each test was made using 0.2 ml. of the plasma or fraction, 0.4 ml. of normal plasma and 0.6 ml. 0.025 M calcium chloride.

Coagulation time of normal plasma 6 min.

that it must be a complex mixture of proteins having various activities in relation to the coagulation mechanism. That it is a complex mixture is borne out by the fact that electrophoretic analysis of chemically prepared euglobulin shows the presence of at least three components (13).

Since so many of the substances active in blood coagulation are euglobulins, it was of interest to determine whether this anticoagulant was contained in the euglobulin fraction of the plasma. Plasma prepared in the usual manner was divided and part of it treated with aluminum hydroxide gel to remove prothrombin. A part of each fraction was then diluted with 10 volumes of distilled

TABLE IX

Activity of euglobulin and supernatant

Time of dialysis	Coagulation time*	
	Supernatant	Euglobulin
days	min.	min.
2	18	7
4	17	6
7	10	6
10	11	7

* Each test was made using 0.2 ml. of the fraction, 0.4 ml. normal plasma and 0.6 ml. of 0.025 M calcium chloride.

Coagulation time of normal plasma 6 min.

water and brought to pH 5.5 by the addition of 1 per cent acetic acid. The precipitates so formed were centrifuged down and dissolved in a volume of 0.15 M sodium chloride equivalent to that of the plasma from which they were derived. The four fractions, namely the untreated plasma, the alumina treated plasma, and the euglobulins derived from them, were tested in the usual manner as shown in Table VIII.

The euglobulins prepared from both the untreated plasma and the alumina treated plasma were found to have no anticoagulant activity. Instead, they both displayed a marked clot accele-

rating action toward normal plasma. This experiment suggested that the anticoagulant was not a euglobulin. Due to the dilution of the supernatant it was not possible to test it for anticoagulant activity. The possibility, therefore, existed that the anticoagulant was a euglobulin but had lost its activity during the procedures involved in the separation of the euglobulin. Euglobulin was accordingly separated by a method which did involve dilution of the supernatant. Plasma was freed of prothrombin by treatment with aluminum hydroxide gel and dialyzed in 5 ml. lots against repeated changes of distilled water. After the times shown in Table IX the precipitates were recovered by centrifuging and dissolved in a volume of 0.15 M sodium chloride equivalent to that of the original plasma. Tests were made on the euglobulins and the supernatants. The supernatants had a definite anticoagulant activity, though not as great as that of the whole plasma. The euglobulins neither accelerated nor retarded the clotting of normal plasma.

Ether extraction of liquid plasma

Plasma obtained in the usual manner was shaken vigorously with one half its volume of ether for 60 seconds. The emulsion so formed was allowed to stand for two hours and then centrifuged. Three layers were formed consisting of ether, plasma-ether emulsion and clear plasma. The plasma layer was removed, freed of ether by evacuation and adjusted to pH 7.4. This ether extracted plasma and the original plasma were compared in their anticoagulant activity against normal plasma. (Table X.)

There was no decrease in the anticoagulant activity of the plasma following ether extraction. From this experiment it is concluded that the active principle of this anticoagulant is not a free lipid, nor it is a loosely bound lipid protein complex such as the antithrombin described by Gruning (14). The possibility remains, however, that

TABLE X

The effect of ether extraction of the liquid plasma

Fraction	Coagulation time*
	min.
Untreated plasma	60
Ether-extracted plasma	50

* Each test was made using 0.2 ml. of the plasma, 0.4 ml. of normal plasma and 0.6 ml. of 0.025 M calcium chloride.

Coagulation time of normal plasma 6 min.

the anticoagulant is a lipoprotein which would not be affected by ether extraction.

DISCUSSION

The properties of the anticoagulants described by Lozner, Jolliffe and Taylor, by Lawrence and Johnson, and in this paper lead to the conclusion that the same substance is present in all three cases. A summary of these properties as found by the three groups of investigators is given in Table XI.

TABLE XI

Characteristics of circulating anticoagulant

Characteristic	Lawrence and Johnson	Lozner, Jolliffe and Taylor	Munro
Prolongs coagulation time of normal blood	yes	yes	yes
Not destroyed by heating	37.5° for 30 hours	61° for 10 min.	65° for 10 min.
Stable to pH	not studied	not studied	6.5 to 11.0
Shows antithrombin activity	no	no	no
Neutralized by protamine	no	no	no
Passes through semi-permeable membrane	no	no	no
Precipitated as euglobulin	not studied	no	no
Precipitated as globulin	not studied	not studied	yes
Extracted by ether	no	no	no

It can be seen that in every case where the same characteristic was studied there is agreement as to the behavior of the anticoagulant.

None of the investigators of this anticoagulant have been able to classify it in any of the groups of potential anticoagulants described by Quick. These groups include antagonists to each of the five factors which participate in the coagulation mechanism. It is apparent therefore that this anticoagulant must fall into one of these groups and failure to identify its type is the result of the inadequacy of the methods available for studying such anticoagulants. With the development of more precise methods it should be possible to determine which phase of the coagulation mechanism is inhibited by this anticoagulant.

The chemical behavior of the substance indicates that the activity is associated with a protein molecule, probably a globulin. This is supported by the fact that the activity is not impaired by dialysis and that on precipitation of the plasma with sodium sulfite the anticoagulant activity is found in the globulin fraction.

SUMMARY

The properties of a circulating anticoagulant found in the blood of a hemophiliac have been in- vestigated. The relation of the anticoagulant to the coagulation mechanism has been studied but its nature has not been determined. Chemically, the anticoagulant activity appears to be associated with the globulin fraction of the plasma.

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STUDIES IN ASCORBIC ACID WITH ESPECIAL REFERENCE TO THE WHITE LAYER. II. THE RELATION OF INTAKE TO BLOOD LEVELS IN NORMAL CHILDREN AND THE EFFECT OF ACUTE AND CHRONIC ILLNESS [1]

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A voluminous literature has accumulated on the effect of various conditions of ascorbic acid intake on plasma levels and urinary excretion in children (1 to 3). Based on these studies, a daily allowance of 50 to 75 mgm. of ascorbic acid has been recommended for children by the National Research Council (4).

Current opinion differs as to whether or not these recommended intakes are in excess of normal requirements. This difference of opinion stems in part from the variability of plasma and urine values as related to intake and vitamin status, but in greater measure from the belief that these values are a reflection of the ascorbic acid concentration in the circulation, rather than the tissue stores (5, 6).

Recent studies have suggested that the level of ascorbic acid in the white cell-platelet layer probably reflects the tissue concentration of ascorbic acid. Butler and Cushman, and others consider that a low level of ascorbic acid in the white layer is a closer index of physiologically significant vitamin C deficiency than plasma or urinary concentration of ascorbic acid (7).

The level of ascorbic acid in the white cell-platelet layer under various conditions of ascorbic acid intake has not been studied extensively. Butler and Cushman observed white layer levels of 25 to 43 mgm. per cent in adults differing in vitamin C nutrition (5). Pijoan and Lozner found that a normal adult could maintain a level of 25 mgm. per cent for a period of months on a daily intake of 25 mgm. of ascorbic acid (6). Crandon, Lund and Dill, and Pijoan and Lozner observed that in adults who were maintained on a vitamin C free diet for 4 to 6 months, the white layer level fell from a normal value of 25 mgm. per cent or more,

to zero, shortly before the appearance of clinical scurvy (8, 9).

In a previous study of a series of ambulatory, non-febrile clinic children whose vitamin C intake was unknown, the range in white layer levels was found to be from 6 to 58 mgm. per cent, with the majority falling between 11 and 30 mgm. per cent (10).

The present investigation supplements the previous study. It includes observations in a series of children whose intake was known. During the course of this study, many of the children experienced intercurrent illness. These observations provided an opportunity to determine the effect of illness on blood levels at various intakes. Observations were made during the course of and convalescence from respiratory infections and childhood diseases. In addition, blood levels of ascorbic acid were determined for a series of rheumatic patients who were under concurrent observation.

MATERIAL AND METHODS

Material: There are represented in this study 76 normal children, ranging in age from 2 to 14 years, with an average age of 7 years, and 40 rheumatic subjects ranging in age from 6 to 15 years, with an average age of 10 years.

Of a total of 164 determinations in the normal group, 40 were made either during or within 2 weeks of intercurrent illnesses, such as respiratory infections, varicella, measles and scarlet fever. In the rheumatic group there were 96 determinations, of which 18 were made during intercurrent illness, and 19 were made during rheumatic activity.

All of these children have been under prolonged medical supervision at the Children's Clinic of the New York Hospital. Many have been under observation from birth. The general nutrition and development of the normal group of children in terms of weight and height distributions at various ages compared favorably with several norms which have been established for children of a better economic status (11 to 13).

Complete dietary histories were obtained at clinic and

home visits on an average of four times a year for a period of several years. In addition, the specific intake of vitamin C was checked each time a blood specimen was taken for analysis. From these records the average daily consumption of protein, fat, carbohydrate and total calories was estimated, using tables of food values (14, 15). It was found that 43 per cent of the children received 80 per cent or more of the recommended allowances of the National Research Council, and were, therefore, considered to have good general diets (4). Forty-one per cent of the children, receiving 60 to 80 per cent of the recommended allowances were considered to have fair general diets, and the remaining 16 per cent, who received less than 60 per cent of the recommended allowances, were considered to have poor general diets.

Only the consumption of citrus fruit and tomato was used for estimating the habitual intake of ascorbic acid, with the addition of 10 mgm. of ascorbic acid assumed to be contributed from all other foods, as suggested by Bessey (2). For some subjects, the vitamin C intake included supplementary tablets of ascorbic·acid.[2]

The data on intake were considered in terms of the total daily amount of vitamin C and the number of mgm. per kgm. body weight. A daily intake of 10 to 40 mgm. was found to be equivalent to 0.5 to 1.9 mgm. per kgm. body weight. In this group, one-third had intakes of more than 25 mgm. For children receiving 50 to 75 mgm. a day, the intake was 1.5 to 2.9 mgm. per kgm. body weight. For children receiving 100 to 200 mgm. a day, the intake was 3.0 to 8.9 mgm. per kgm. body weight. On intakes of 200 to 600 mgm., the daily dosage was invariably 9 mgm. per kgm. or more.

When changes in intake were made, it was found that the white layer levels became stabilized within one month following the change, and therefore, all intakes of one month's duration or more were considered habitual.

Methods: Serial blood specimens were collected over a period of 3 to 10 months, 2 to 3 hours following a vitamin C free breakfast.[3] The number of specimens collected from each child ranged from 1 to 9. In 40 per cent of the children, at least 3 specimens were collected.

The modified phenylhydrazine method of Roe and Kuether, which has been described previously, was closely followed for these analyses, (10). Four ml. of venous blood were collected in a tube containing dry mixed oxalates, care being taken to avoid hemolysis. Since it was found that both plasma and white layer specimens could be stored frozen for a period of at least two weeks without loss of ascorbic acid, the specimens were usually collected and centrifuged on one day, and analyzed several days later. The white layer specimens contained all of the platelet layer, and about 50 per cent of the leukocyte layer. No specimen weighed less than 5 mgm., and specimens in which there was possible contamination by red

cells were discarded. An experimental error of ± 3 mgm. per cent was established for this method of white layer analysis (10). This range of error was confirmed in the present study by frequent analysis of duplicate and triplicate specimens from the same individual.

The stability of the white layer level following recent changes in intake is indicated by the following tests. Fasting determinations were made on 7 convalescent children on the pediatric pavilion of the New York Hospital prior and subsequent to an increase in intake of 500 mgm. daily for three days. Fasting specimens were then collected on the fourth, fifth and sixth days after resumption · of the habitual intake. In the 21 subsequent determinations, the variation for each child was within the experimental error of the method. In only one child was there an elevation to 31 mgm. per cent in the white layer from a control level of 24 mgm. per cent. In 3 additional subjects who received 1000 mgm. in 10 divided doses for one day, the fasting white layer level, prior and subsequent to this increase in intake, differed by less than 3 mgm. per cent.

The possible diurnal fluctuations in the white layer level were also considered. In 51 determinations at 2-hour intervals during a 24-hour period on 8 volunteers from the house staff, it was found that 15 determinations varied by more than 4, but less than 10 mgm. per cent. These variations might be attributed to the possible traumatic effect of multiple venepunctures, although hemolyzed specimens were discarded. Varying degrees of physical activity during the day might also have been a contributing factor. It is noteworthy that the fasting white layer levels on the day following the test did not differ from the initial fasting levels by more than 1 mgm. per cent.

OBSERVATIONS ON NORMAL CHILDREN

Distribution of blood levels of ascorbic acid: In Figure 1 ascorbic acid levels in the white layer at specific plasma levels are presented. It will be noted that at plasma levels of 0.4 mgm. per cent or less, there is a direct correlation. At higher plasma levels, high and low white layer levels are obtained.

At specific levels of intake of ascorbic acid, less than 9 mgm. per kgm. body weight, there is a closer correlation between plasma and white layer levels. In Figure 2A it may be observed that on intakes of 0.5 to 1.9 mgm. per kgm. body weight, representing for two-thirds of the subjects an intake of 15 to 25 mgm. a day, and for one-third of the subjects an intake of 25 to 40 mgm. a day, the majority of the plasma levels are 0.4 mgm. per cent or ·less, and two-thirds of the white layer levels are 20 mgm. per cent or less. It is noteworthy that 90 per cent of the white layer levels are less than 25 mgm. per cent.

In Figure 2B it may be noted that on intakes of 1.5 to 2.9 mgm. per kgm. body weight, representing the average allowance of 50 to 75 mgm. a day formulated by the National Research Council, most of the plasma levels are 0.7 mgm. per cent or more. The majority of the white layer levels are 25 mgm. per cent or more.

In Figure 2C it may be noted that an intake of 3.0 to 8.9 mgm. per kgm. body weight, representing a daily dosage of at least 100 mgm. of ascorbic acid, but less than 200 mgm., the blood levels are not significantly different from the values obtained on intakes of 50 to 75 mgm. a day.

It is of particular significance that intakes of 9 mgm. per kgm. body weight or more, representing massive dosages for at least one month of 200 to 600 mgm., the white layer level is usually less than 25 mgm. per cent (Figure 2D). It may also be noted that the plasma levels in 14 of the 33 determinations were 1.4 mgm. per cent or less, although the majority were more than 0.7 mgm. per cent. These observations were not anticipated.

To check the validity of these observations, two members of the staff, whose white layer levels had been determined at frequent intervals for one year, volunteered to take daily divided doses of 600 to 900 mgm. of ascorbic acid, in addition to their usual intake of 100 to 150 mgm. a day.

In one subject, the white layer level fell from a control level of 35 mgm. per cent to 18 mgm. per cent, with a plasma level of 1.0 mgm. per cent after a total daily intake of 700 to 750 mgm. for 3 weeks. Two weeks later the white layer level was 22 mgm. per cent on the same intake. A daily intake of 100 to 150 mgm. was then resumed, and in 3 weeks, the white layer level was 36 mgm. per cent, and the plasma level was 1.4 mgm. per cent.

In the second subject, the white layer level fell from a control level of 40 mgm. per cent to 31 mgm. per cent, with a plasma level of 1.7 mgm. per cent 2 weeks after a total daily intake of 700 to 750 mgm. had been established. The white layer level fluctuated between 30 and 34 mgm. per cent in the next 5 weeks, during the last 3 of which the intake was increased to 1000 to 1050 mgm. a day. After 4 weeks on the increased dosage, the white layer level was 16 mgm. per cent, with a plasma level of 0.7 mgm. per cent. The 24-hour urinary excretion was then determined, and it was found that 600 mgm. were excreted on one day, and 941 mgm. were excreted 3 days later. Two weeks after the resumption of a daily intake of 100 to 150 mgm. the white layer level was 32 mgm. per cent, the plasma level 1.1 mgm. per cent, and the urinary excretion was 38 mgm.

It is of interest that in both subjects, the total and differential white counts remained within normal limits.

COMMENT

It is apparent that intakes of approximately 25 mgm. a day will not consistently maintain a level of 25 mgm. per cent in the white layer of well child-
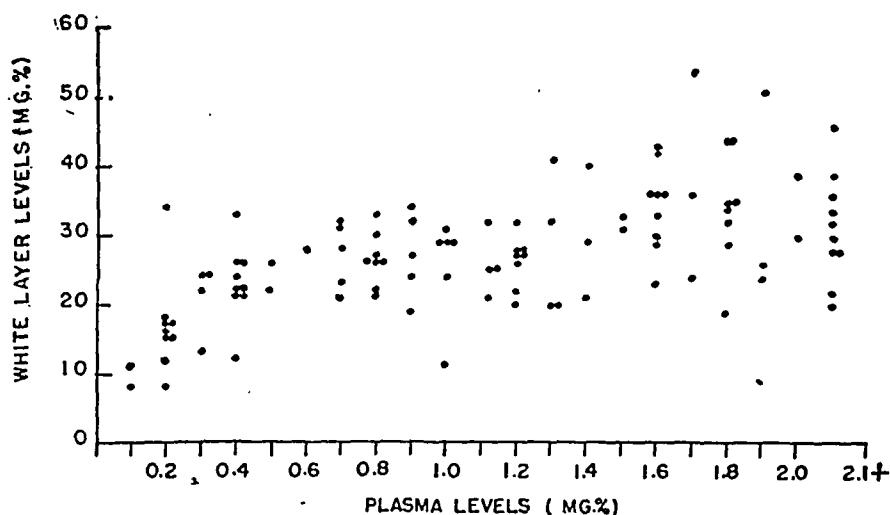
FIG. 1. ASCORBIC ACID LEVELS IN PLASMA AND WHITE LAYER IN NORMAL CHILDREN ON INTAKES OF 0.5 TO 8.9 MGM. PER KGM. BODY WEIGHT (15 TO 200 MGM. OF ASCORBIC ACID A DAY)
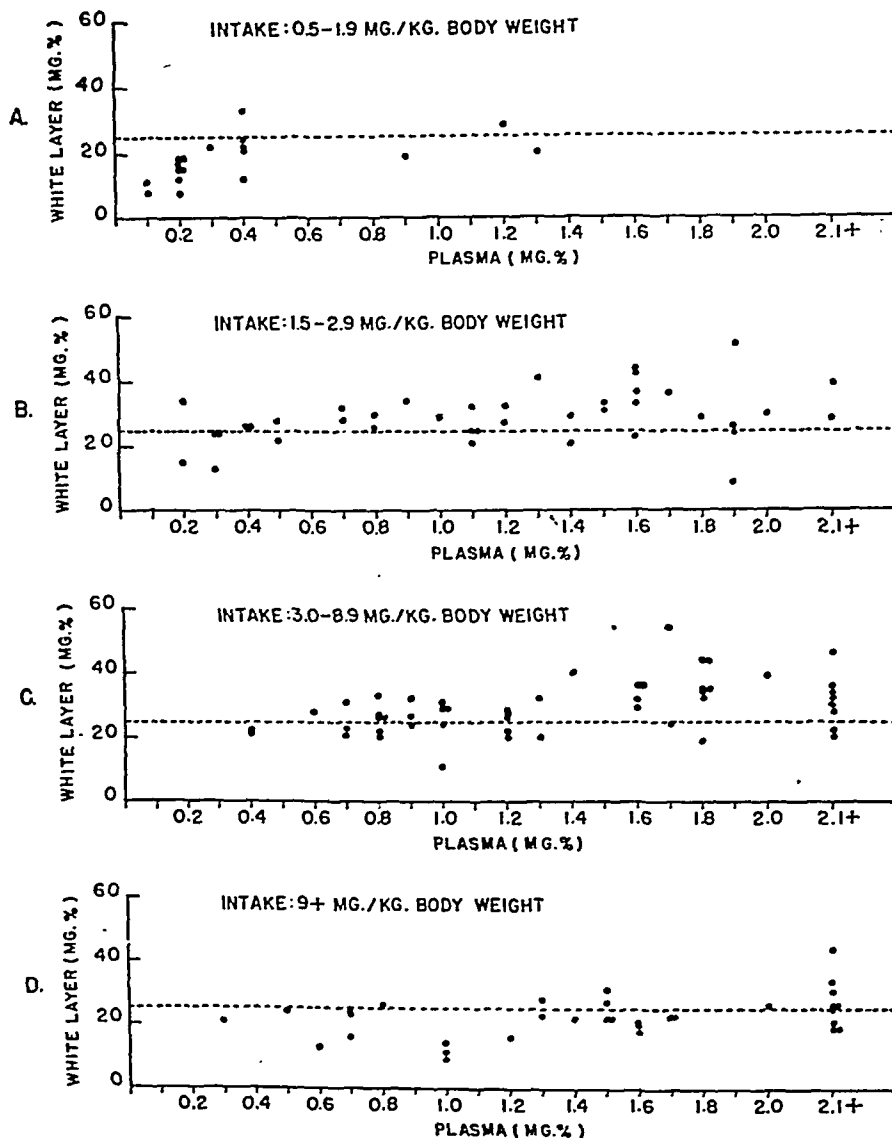
FIG. 2. PLASMA AND WHITE LAYER LEVELS AT VARIOUS INTAKES OF ASCORBIC ACID

A. Intakes of 15 to 40 mgm. a day.

B. Intakes of 50 to 75 mgm. a day.

C. Intakes of 100 to 200 mgm. a day.

D. Intakes of 200 to 600 mgm. a day (including 16 normal subjects and 17 well rheumatic subjects).

ren. This is contrary to the observations by Pijoan and Lozner in an adult (8).

Of particular interest is the observation that the majority of children receiving the recommended allowance of 50 to 75 mgm. a day, formulated by the National Research Council, maintained levels of 25 mgm. per cent or more during apparent health.

It might be questioned whether intakes sufficient to maintain a level of 25 mgm. per cent are necessary. We have obtained no evidence to indicate that children with such levels had any advantage over others with lower levels. The small number of children on low intakes of vitamin C were also those who had inadequate diets. Among these children, poor nutrition and frequent illness could

not be attributed to a specific inadequacy of vita-min C intake. However, it is reasonable to assume that concomitant with the gradually lowering level of ascorbic acid in the white layer in the weeks preceding manifest scurvy, there are early tissue changes. It would therefore, seem desirable to provide an intake of ascorbic acid sufficient to maintain the white layer level at 25 mgm. per cent or more. It should be borne in mind that dietary surveys of school children have reported that the majority of children do not receive an orange or its equivalent every day.

It is apparent that massive dosage of ascorbic acid, given over a period of one month or more to children whose previous intake was adequate, is not advisable. Unless the previous level is low, intakes of 9 mgm. per kgm. body weight or more often depress the white layer level below 25 mgm. per cent. In addition, several children did not appear to tolerate massive dosage over a prolonged

period. Anorexia, abdominal pain and erythematous eruptions were noted, which could not be attributed to other factors (Table I).

OBSERVATIONS ON CHILDREN WITH INTERCURRENT ILLNESS

Distribution of blood levels of ascorbic acid: The previous observations represent blood levels of ascorbic acid in children who were in apparent good health for the preceding two weeks or more. In Table IIB are summarized the white layer levels for children on various intakes, who experienced respiratory infections or childhood diseases within two weeks of the time of analysis. Of particular importance is the fact that lower levels are observed on intakes of 1.5 to 2.9 mgm. per kgm. body weight than for well children on the same intakes (Table IIA). On intakes of 3.0 to 8.9 mgm. per kgm. body weight, the majority of the levels are above 25 mgm. per cent. It is also of interest that

TABLE I

The response of plasma and white layer levels of ascorbic acid to various intakes in a representative group of cases

Sub-ject	Age (years)	Average Weight (kgm.)	Serial determinations								
			1	2	3	4	5	6	7	8	9
A. Non-Rheumatic Subjects:											
P.S.	8	29	11/4/44	12/9/44	1/6/45	3/31/45	6/16/45				
Plasma (mgm. per cent)			1.7	2.1	1.8	1.4	2.2				
White Layer (mgm. per cent)			54	31	44	40	26				
Intake (mgm. per kgm. body wt.)			3.7	5.4	7.0	7.0	21.6				
Duration of Intake			Habitual	1 month	1 month	3 months	1 month				
Remarks			Well	Febrile	Well	Well	Well				
E.L.	2	13	11/25/44	2/24/45	5/26/45	6/23/45					
Plasma (mgm. per cent)			0.1	1.8	1.0	1.0					
White Layer (mgm. per cent)			8	34	31	25					
Intake (mgm. per kgm. body wt.)			0.9	8.0	7.2	35.0					
Duration of Intake			Habitual	3 months	3 months	1 month					
Remarks			Well	Well	Well	Febrile (U.R.I.) Erythema					
P.B.	8	29	11/18/44	12/28/44	2/17/45	5/19/45	6/29/45				
Plasma (mgm. per cent)			0.7	2.4	1.6	1.7	2.1				
White Layer (mgm. per cent)			32	34	30	24	19				
Intake (mgm. per kgm. body wt.)			1.9	3.7	3.5	4.5	18.0				
Duration of Intake			2 weeks	1 month	2 months	3 months	1 month				
Remarks			Well	Well	Febrile (U.R.I.)	Well	Well				
E.Q.	6	20	1/6/45	2/24/45	3/24/45	5/26/45	6/27/45				
Plasma (mgm. per cent)			1.2	0.7	0.5	0.7	1.3				
White Layer (mgm. per cent)			27	24	20	23	23				
Intake (mgm. per kgm. body wt.)			3.8	10.0	7.6	15.0	24.6				
Duration of Intake			Habitual	2 months	1 month	2 months	1 month				
Remarks			Well	Well	Febrile (U.R.I.)	Well	Well				
T.S.	6	20	11/25/44	1/29/45	4/14/45	6/27/45					
Plasma (mgm. per cent)			1.9	1.6	1.7	1.6					
White Layer (mgm. per cent)			26	36	33	20					
Intake (mgm. per kgm. body wt.)			2.7	5.2	5.0	26.0					
Duration of Intake			Habitual	2 months	2 months	1 month					
Remarks			Well	Well	Well	Well					
D.S.	4	14	11/25/44	1/29/45	4/14/45	6/27/45					
Plasma (mgm. per cent)			1.8	1.2	2.0	2.1					
White Layer (mgm. per cent)			29	26	34	19					
Intake (mgm. per kgm. body wt.)			3.5	6.9	6.8	33.5					
Duration of Intake			Habitual	2 months	2 months	1 month					
Remarks			Well	Well	Well	Well					

TABLE I—*Continued*

Sub-ject	Age (years)	Average Weight (kgm.)	Serial determinations								
			1	2	3	4	5	6	7	8	9
B. Rheumatic Subjects:											
E.Q.	8	28	1/6/45	2/24/45	3/24/45	5/26/45	6/27/45				
Plasma (mgm. per cent)			1.2	0.8	1.1	0.6	1.2				
White Layer (mgm. per cent)			27	31	27	13	16				
Intake (mgm. per kgm. body wt.)			11.6	13.5	15.8	10.5	17.3				
Duration of Intake			2 months	2 months	1 month	2 months	1 month				
Remarks			Febrile Chorea	Chorea	Febrile Chorea	Well	Well				
G.E.	15	63	2/28/45	3/3/45	3/17/45	4/14/45	5/19/45	6/9/45			
Plasma (mgm. per cent)			0.8	1.6	1.6	1.3	1.3	1.3			
White Layer (mgm. per cent)			32	31	35	26	21	28			
Intake (mgm. per kgm. body wt.)			1.2	17.1	10.0	4.8	4.5	8.7			
Duration of Intake			1 month	1 day	1 month	1 month	1 month	3 weeks			
Remarks			Active Rh.	Active Rh.	Active Rh.	Active Rh.	Well	Well			
H.A.	10	32	11/11/44	12/16/45	2/17/45	5/19/45	5/29/45	6/9/45			
Plasma (mgm. per cent)			2.4	1.8	2.1	1.1	1.4	1.5			
White Layer (mgm. per cent)			44	41	39	22	23	27			
Intake (mgm. per kgm. body wt.)			12.9	10.9	8.5	11.7	11.7	9.3			
Duration of Intake			Habitual	1 month	2 months	3 months	2 weeks	2 weeks			
Remarks			Well	Recent U.R.I.	Well	Erythema	Erythema Abdominal Pain	Well Abdominal Pain			
M.H.	13	45	11/11/44	12/28/44	2/10/45	2/17/45	3/24/45	4/21/45	5/8/45	5/26/45	
Plasma (mgm. per cent)			0.2	0.9	0.9	0.6	1.7	1.2	0.6	0.5	
White Layer (mgm. per cent)			12	42	29	17	26	26	15	24	
Intake (mgm. per kgm. body wt.)			0.4	12.8	3.5	3.5	13.0	13.5	14.2	14.2	
Duration of Intake			Habitual	1 month	2 months	1 week	1 month	1 month	2 weeks	2 weeks	
Remarks			Febrile (U.R.I.)	Febrile (U.R.I.)	Active Rh.	Active Rh.	Active Rh.	Febrile (U.R.I.)	Recent U.R.I. Petechiae 2 weeks	Well (Petechiae 2 months later)	
J.S.	11	35	4/14/45	4/28/45	6/27/45						
Plasma (mgm. per cent)			1.0	1.2	1.0						
White Layer (mgm. per cent)			29	26	11						
Intake (mgm. per kgm. body wt.)			2.9	11.5	8.2						
Duration of Intake			2 weeks	2 weeks	2 months						
Remarks			Well	Well	Well						
W.K.	8	31	12/16/44	2/24/45	3/24/45	6/2/45					
Plasma (mgm. per cent)			1.9	1.7	1.3	2.1					
White Layer (mgm. per cent)			40	32	34	26					
Intake (mgm. per kgm. body wt.)			6.3	6.3	6.4	13.0					
Duration of Intake			1 month	2 months	1 month	2 months					
Remarks			Well	Well	Well	Well					

on massive dosage a greater number of white layer levels are above 25 mgm. per cent than in the group in apparent good health.

Blood levels in rheumatic patients: In Table IIIA, it may be noted that there is no significant difference in the blood levels on various intakes for rheumatic subjects in apparent good health, in comparison to well, non-rheumatic subjects (Table IIA). There were, however, more white layer levels above 25 mgm. per cent in the group receiving massive dosage than in the comparable non-rheumatic group.

Although the number of observations of rheumatic subjects during intercurrent illness is limited, it may be observed in Table IIIB that the distribution of blood levels is similar to that observed for the comparable non-rheumatic group. In several children on low intakes the white layer levels were 10 to 15 mgm. per cent.

For the few subjects who experienced an acute

rheumatic episode, it may be noted in Table IIIC that the majority of the white layer levels are lower at all levels of intake. In several rheumatic patients who had received massive dosage of ascorbic acid over a period of months, anorexia, abdominal pain and petechiae were noted (Table I).

DISCUSSION

The difficulties inherent in the assessment of dietary histories were minimized in this study. The families were under close medical supervision over a prolonged period of observation, during which time there was ample opportunity to estimate the accuracy and reliability of the dietary records. Corroborative evidence is given by the consistency of the blood levels of ascorbic acid at any specific intake. It was observed that within a family, recorded differences in ascorbic acid intake for each child were consistent with the levels of ascorbic acid in the white layer. The majority

TABLE II

White layer levels at various intakes in normal children during health and intercurrent illness

Intake (mgm. per kgm. body wt.)	Distribution of white layer levels (mgm. per cent)					Total observations
	<15	16 to 20	21 to 24	25 to 35	36+	
A. Normal subjects						
0.5 to 1.9	7	5	4	2		18
1.5 to 2.9	2	1	7	23	7	40
3.0 to 8.9	1	4	11	25	9	50
.....9.0+	1	5	6	4		16
B. Normal subjects with intercurrent illness						
0.5 to 1.9	3	1				4
1.5 to 2.9	1	1	3	5		10
3.0 to 8.9		1	6	12		19
.....9.0+		1	1	5		7

of the children whose general diet was estimated as adequate were of good nutrition and compared favorably with a series of observations on normal children of a higher economic status.

It has been shown that a single white layer specimen is reliable from the standpoint of chemical analysis. It is evident that a fasting specimen is preferable, since some diurnal fluctuation was observed in serial specimens. Since the majority of the serial determinations did not differ by more than 4 mgm. per cent, the fluctuations observed were interpreted to be of no clinical significance.

The observations on the relation of intake of ascorbic acid to the level in the white layer are interpreted to indicate that the recommended allowance of 50 to 75 mgm. a day formulated by the National Research Council is not excesive. When this intake represents a dosage of 1.5 to 2.9 mgm. per kgm. body weight, levels of 25 mgm. per cent were maintained by children who were in apparent good health.

It is of interest that Roberts and Roberts (16), in an intensive study of 5 children, observed that on a range in intake of 1.7 to 2.4 mgm. per kgm. body weight, representing 65 to 75 mgm. a day of pure ascorbic acid, a plasma level of 0.7 mgm. per cent was maintained. This intake was sufficient to provide for an average retention value and to produce a 50 per cent excretion of a test dose. The findings of Bessey and White (2) in a series of ambulatory children on an estimated intake of

about 50 mgm. a day, are in essential agreement. The majority of the well children in this study on intakes of 50 mgm. a day or more maintained plasma levels of 0.7 mgm. per cent or more. It is probable that when there is no recent change in vitamin C intake, both plasma levels and urinary excretion reflect the intake of ascorbic acid. However, it was observed that following recent changes in intake, plasma and urine values varied significantly, while the white layer level remained constant. Since white layer levels do not vary following recent changes in intake, it would appear that the white layer level is a more reliable criterion of habitual intake of ascorbic acid than plasma or urine values.

The tendency to consider vitamin C requirements only from the standpoint of the prevention of scurvy would not appear advisable for children. If the level in the white layer reflects in any measure the tissue concentration of ascorbic acid, an intake sufficient to insure the maintenance of normal levels should be given. On intakes of less than 40 mgm. a day, the white layer levels approached the prescorbutic values observed in experimentally induced scurvy.

It has been shown that during the course of acute or chronic illness, or during convalescence,

TABLE III

White layer levels at various intakes in rheumatic subjects

Intake (mgm. per kgm. body wt.)	Distribution of white layer levels (mgm. per cent)					Total observations
	<15	16 to 20	21 to 24	25 to 35	36+	
A. Well rheumatic subjects						
0.5 to 1.9	3	3	1	1		8
1.5 to 2.9		1	3	3		7
3.0 to 8.9	1	2	4	13	7	27
.....9.0+	3	1	6	6	1	17
B. Rheumatic subjects with intercurrent illness						
0.5 to 1.9	2	2				4
1.5 to 2.9	1			2		3
3.0 to 8.9		2		2	1	5
.....9.0+	2			2	2	6
C. Rheumatic subjects with acute rheumatic fever						
0.5 to 1.9				1	2	4
1.5 to 2.9	1	1	1	3	1	7
3.0 to 8.9	1		1	5		8
.....9.0+	1		2			

blood levels were lowered. The mean white layer level on daily intakes of 1.5 to 2.9 mgm. per kgm. body weight (50 to 75 mgm.) was 24.2 compared to 28.9 for well children. While intakes of 1.5 to 2.9 mgm. per kgm. body weight will maintain normal levels during health, it would seem desirable to insure an adequate margin of reserve by providing larger intakes, since most children have repeated illnesses. It has been observed that during acute and chronic illness, an intake of at least 100 mgm. a day will maintain white layer levels above 25 mgm. per cent. Such an intake would seem to be particularly indicated for growing children, experiencing frequent febrile illnesses, and for those suffering from chronic diseases, such as rheumatic fever. No evidence was obtained to indicate that the requirements for rheumatic fever differ from those in other acute febrile illnesses.

The observed depression of the level of ascorbic acid in the white layer on intakes of 9 mgm. per kgm. or more, is of considerable interest, but difficult or impossible to interpret at the present time. The excess of ascorbic acid over body requirements may be stored, destroyed, or excreted unchanged. The variability of the urinary output makes it difficult to account for the total amount ingested on the basis of the plasma, white cell and urinary content of ascorbic acid. It is probable that varying amounts of ascorbic acid are destroyed, and the depression of the white layer level after prolonged massive dosage suggests that such destruction may be carried beyond normal limits. In this connection, it is of interest to note that the fasting plasma level rises as high as 2.3 mgm. per cent early in the course of massive dosage, but by the time the white layer level is lowered, the fasting plasma level is usually between 0.7 and 1.0 mgm. per cent. It is possible that the normal renal threshold may be lowered after prolonged therapy.

Although it has generally been observed that crystalline ascorbic acid is non-toxic in moderate doses, there have been reports of vagotonic symptoms in growing children at the height of vitamin C action (17). Fatigue, anorexia, increased peristalsis, dermography and erythema have been attributed to sensitivity or idiosyncrasy to ascorbic acid. Comparable symptoms were noted in several children in our series who were on massive dosage. Randoin has noted that if a scorbutic animal is given relatively larger quantities of vitamin C the animal may lose weight, and the symptoms of scurvy reappear (17).

It has generally been assumed that the amount of ascorbic acid ingested in excess of body requirements is excreted, and is therefore wasteful. Some evidence has been presented that massive dosage of ascorbic acid may be detrimental. It would, therefore, seem inadvisable to administer massive doses of ascorbic acid over a prolonged period of time in children whose previous intake has been adequate.

SUMMARY

1. In normal children, the range in plasma levels of ascorbic acid varied between 0.1 and 2.1 mgm. per cent, and the white layer levels ranged between 8 and 54 mgm. per cent on intakes of ascorbic acid of 0.5 to 8.9 mgm. per kgm. body weight, (15 to 200 mgm. a day).

2. On habitual daily intakes of 0.5 to 1.9 mgm. per kgm. body weight (average intake of about 25 mgm.), the plasma levels were consistently 0.4 mgm. per cent or less, and the white layer levels were 20 mgm. per cent or less.

3. On habitual daily intakes of 1.5 to 2.9 mgm. per kgm. body weight (average intake 50 to 75 mgm.), the majority of the plasma levels were 0.7 mgm. per cent or more, and the white layer levels were 25 mgm. per cent or more in well children.

4. On daily intakes of 9 mgm. per kgm. body weight or more (200 to 600 mgm. a day) for a period of at least one month, the majority of the white layer levels were less than 25 mgm. per cent, and one-third of the plasma levels was less than 1.4 mgm. per cent.

5. During the course or convalescence from intercurrent febrile illness, intakes of 3.0 to 8.9 mgm. per kgm. body weight (100 to 200 mgm. a day) were necessary to maintain white layer levels of 25 mgm. per cent or more.

6. Blood levels in rheumatic subjects did not differ from those found in comparable non-rheumatic subjects on the same intakes.

CONCLUSIONS

1. The recommended daily allowance of 50 to 75 mgm. of ascorbic acid for children formulated by the National Research Council is not excessive.

2. To insure the maintenance of levels of 25 mgm. per cent or more in the white layer during childhood, habitual intakes of 3.0 to 8.9 mgm. per kgm. body weight (at least 100 mgm. a day) are necessary.

3. Habitual intakes of 9 mgm. per kgm. body weight (200 to 600 mgm. a day) or more are unnecessary and inadvisable.

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THE EFFECT OF CLOTHING ON THE ABILITY OF MEN TO WORK IN INTENSE HEAT

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Although many observations have been made on the upper limits of heat tolerated by working men (1 to 6), little work has been published on the specific effects of clothing on man's performance in these limiting hot environments. Gagge, Winslow, and Herrington's calorimetric studies (7) on the effect of clothing were done on resting men in environments with operative temperatures below 101° F. However, Robinson, Turrell, and Gerking (8) have studied this problem by comparing the most severe environments in which both nude and clothed men could maintain thermal equilibrium after the second hour of a six hour work period. They showed experimentally that men wearing a single layer of light clothing (windbreak poplin) could maintain thermal equilibrium only when the environments were less severe than those tolerated by nude men. The clothing had the same effect as lowering the limiting wet bulb temperature of the environment 2° to 6° F., depending on the work rate and environment.

A previous report from this laboratory (6) described the upper limits of heat that could be tolerated by highly acclimatized nude men working for four hours at approximately 250 Cal. per hour. The present investigation was undertaken to extend these data and to study the rôle of clothing by determining certain of the most severe environments in which highly acclimatized clothed men could work at this rate.

PROCEDURE

All experiments were conducted during January, February and March 1945 in a laboratory hot room (35' × 22' × 14'). A Carrier 15–T6 air processing unit permitted control to within + or − 1° F. of the desired dry or wet bulb air temperature. For each set of conditions the air temperature was maintained for four to five days before any tests were conducted so that walls and air were

in equilibrium. Throughout the tests, the dry and wet bulb temperatures were measured every fifteen minutes, by means of motor driven psychrometers carried around the track at a level of four feet. Air temperatures showed a gradient of about 4 degrees from the floor to the six foot level. Wall temperatures were approximately 2° to 5° cooler than the air, floor temperatures 10° cooler at a dry bulb temperature of 95° F. and up to 20° cooler at 120° F. The air movement was turbulent and was essentially that produced by the movement of the men marching at 3 mph.

In the zone of hot environments in which the dry bulb temperature (D.B.) ranges from 90° to 120° F. man's performance is limited by the wet bulb temperature (W.B.) (6). Accordingly, it was felt that the upper limits of working men's tolerance to heat could be determined by studying man's ability to march for four hours under varying wet bulb temperatures at two levels of dry bulb temperature. After preliminary study the following test environments were chosen because they represent critical wet bulb temperatures.

D.B. (°F)	W.B. (°F)	R.H. (per cent)
95	94	97
93	92	97
120	92	35
120	90	31
120	88	25

Ten healthy male soldiers (Table I) were the subjects. After a two-week physical conditioning program in the cool, the men were acclimatized to the heat by graded

TABLE I

Physical characteristics of subjects

Name	Age	Weight	Height	Surface area
	yrs.	lbs.	in.	m²
Di	21	140	68	1.75
Hi	24	150	67	1.78
Ka	24	171	71	1.97
Kn	20	168	72	1.99
Li	20	169	72	1.99
Lo	20	141	64	1.69
Ma	20	153	70	1.86
Mi	20	145	69	1.80
Sc	21	178	71	2.02
Sz	23	144	69	1.80
Mean	21	156	69	1.87

activity at D.B. 120°—W.B. 88° F. for 21 days before the test began. They were fully acclimatized to four hours of marching with pack in this environment before any of the test environments were studied. In addition to this preliminary acclimatization, the men marched for 3 days in each new environment, before the testing was done. This added acclimatization to each specific environment was necessary to insure peak performance at these limiting environments which did impose a greater thermal load than D.B. 120°—W.B. 88° F. At each environment the nude and clothed state were compared in each man on two consecutive test days. The standard work consisted of marching with 20 lb. packs, at 3 mph. for four hours continuously around a 77 foot track in the hot room. This work rate was previously determined on other subjects to be approximately 250 Cal. per hour. The four work periods each morning were followed by a three hour rest in the nude, in the afternoon. At night the men lived in barracks maintained at about 70° F.

Each man was studied (1) in the nude, wearing only shoes and socks, (2) clothed in a standard two piece single layer herringbone twill (8 oz.) army fatigue uniform, and (3) clothed in a special impregnated herringbone twill uniform. This latter garment was treated with an impregnating mixture containing paraffin, which increased the weight of the uniform by 40 per cent, increased its water repellency and reduced its permeability to air, water, and water vapor. The uniforms were dry at the start of each day. The clothing was always worn with cotton shorts, socks and shoes. The trouser legs were tucked inside the socks, the jacket of shirt inside the trousers, and the clothing always completely buttoned.

The subjects entered the hot room to be weighed nude and to dress. After an initial 8 minutes the skin temperatures, pulse rate (standing erect) and rectal temperature were taken. The start of the march immediately followed. Pace discipline was enforced. The heart rate (marking time) and rectal temperature were taken again at the end of each hour, during a three minute break. At the end of the four hour march, or at the time of falling out, skin temperatures, pulse rate and rectal temperature were again taken, as well as a final nude weight. Water consumption (0.1 per cent saline, 95° F.) and urine output were recorded. Clothing was weighed before and immediately after each wearing. Skin temperatures of the cheek, chest, palm, forearm, and calf were determined radiometrically on the erect subject, the clothing being pushed away just sufficiently to permit placing the radiometer. The temperatures were integrated into an average skin temperature by the following weighting formula based on that of Hardy and Dubois (9): cheek 0.10, chest 0.44, forearm 0.14, calf 0.23, palm 0.09. Only the average weighted skin temperature is reported. Rectal temperatures were measured with calibrated clinical thermometers, pulse rates by palpation. Records were kept of the symptoms, complaints, general appearance, vigor and alertness of the men. Men were removed from test only when objective or subjective signs indicated it to be necessary.
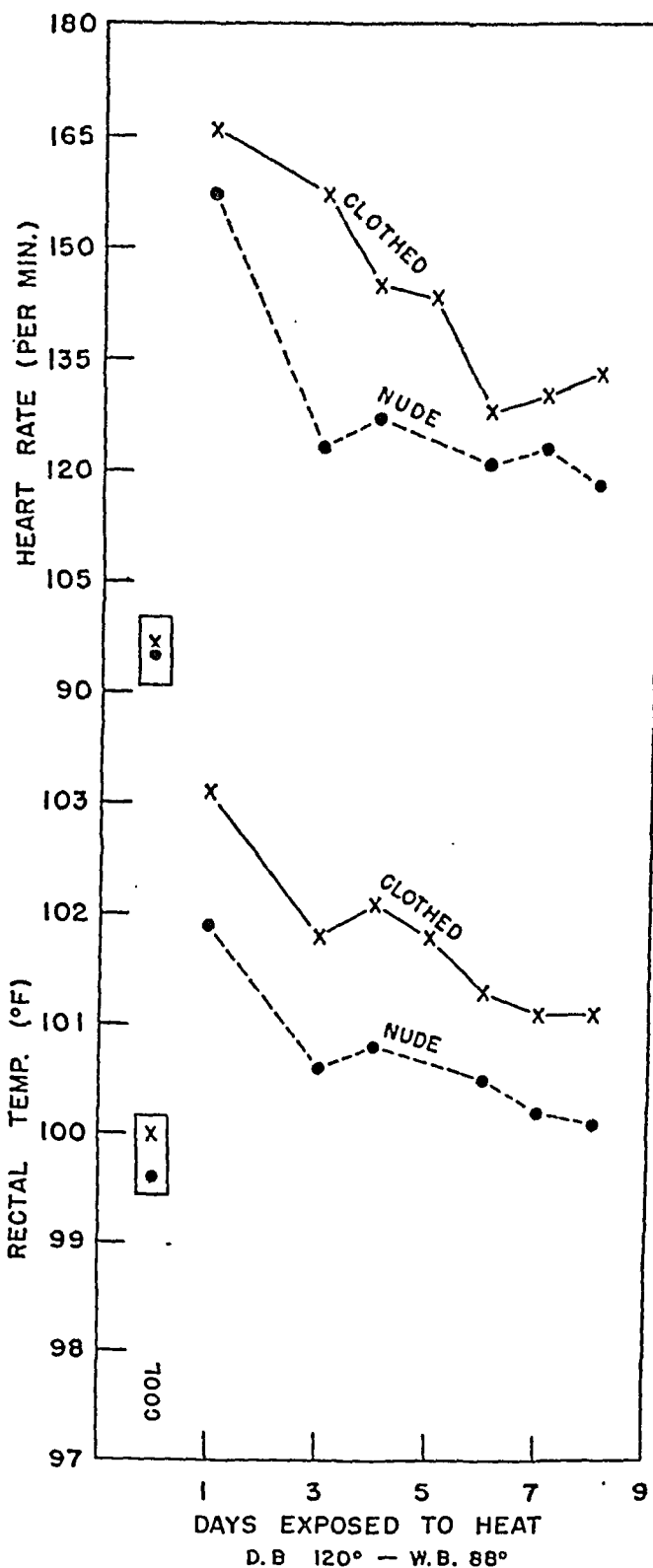
Fig. 1. Effect of Wearing Herringbone Twill Uniform on the Rate of Acclimatization

Data presented were obtained at the end of 1 hour march at D.B. 120°—W.B. 88° F. except that the data in blocks were taken on men working at D.B. 77°—W.B. 62° F.

TABLE II

Physiologic effects of wearing standard herringbone twill clothing on acclimatized men working in the heat
D.B. 120° F—W.B. 90° F

Name	Clothing	Rectal temperature °F					Pulse rate per min.					Skin temp. (Avg. wtd.) °F		Weight loss* (Sweat)
		0	1	hours 2	3	4	0	1	hours 2	3	4	hours 0	4	grams per hour
Hi	Nude	98.7	99.7	100.0	99.9	99.9	108	123	111	111	117	96.5	96.4	1477
	Clothed	99.3	100.6	101.0	100.1	100.0	102	117	126	123	117	97.1	97.3	1859
Ka	Nude	98.6	99.7	99.8	99.7	99.8	102	123	123	120	114	96.3	97.2	1421
	Clothed	98.4	100.4	100.7	100.3	100.0	111	135	129	123	132	97.3	97.2	1711
Lo	Nude	98.4	99.6	100.2	99.9	100.2	96	114	120	120	117	96.5	97.8	1645
	Clothed	98.4	100.7	101.2	100.9	100.8	96	120	129	132	135	97.3	97.9	1543
Mi	Nude	98.5	100.0	100.5	100.4	100.3	117	126	129	123	123	97.6	96.8	1901
	Clothed	98.3	100.7	101.2	101.0	101.0	102	120	153	138	138	97.3	97.3	2123
Sc	Nude	99.0	100.4	100.3	99.9	100.1	111	129	126	123	123	97.7	96.9	2239
	Clothed	98.7	100.8	100.6	100.3	100.2	105	123	135	126	120	98.2	97.1	2693
Mean	Nude	98.6	99.9	100.2	100.0	100.1	107	123	122	119	119	96.9	97.0	1737
	Clothed	98.6	100.6	100.9	100.5	100.4	103	123	134	128	128	97.4	97.4	1986

* The term weight loss is used to indicate gross sweat loss only. It does not represent an actual change in body weight since throughout all tests the men maintained body weights constant to within ± 0.5 kgm. by water replacement.

RESULTS

During the initial period of the three weeks' acclimatization to D.B. 120°—W.B. 88° F., the ten men were divided into two groups to study the effect of clothing on acclimatization. One group wore herringbone twill uniforms each day and the other worked nude. During the first eight days the work load which started with one hour was increased by an hour every third day until the standard work period of four hours was reached. Only the man's rectal temperatures and pulse rates at the end of the first hour were stud-

TABLE III

Physiologic effects of wearing standard herringbone twill clothing on acclimatized men working in the heat
D.B. 120° F—W.B. 92° F

Name	Clothing	Rectal temperature °F					Pulse rate per min.					Skin temp. (Avg. wtd.) °F		Weight loss (Sweat)
		0	1	hours 2	3	4	0	1	hours 2	3	4	hours 0	4	grams per hour
Hi	Nude	98.4	100.4	100.4	100.4	100.6	87	111	102	117	111	97.2	98.7	1562
	Clothed	98.2	100.8	101.7	101.7	102.0	102	129	111	147	123	97.9	99.4	1688
Ka	Nude	98.6	100.0	100.0	100.2	100.7	96	120	120	132	123	97.1	98.5	1684
	Clothed	98.7	101.2	102.0	102.0	101.8	105	150	147	141	150	97.2	99.3	1774
Lo	Nude	98.6	100.1	101.4	101.7	102.1	102	144	129	120	132	98.1	99.4	1396
	Clothed	98.9	102.0	103.1	*		105	144	144	*		98.0	*	*
Mi	Nude	98.8	100.3	100.4	100.5	101.0	105	126	150	123	126	97.8	98.7	2071
	Clothed	98.8	101.3	101.8	†		129	147	141	†		98.3	†	†
Sc	Nude	99.3	101.4	101.2	101.2	101.5	111	156	123	120	126	97.8	98.2	2543
	Clothed	99.0	102.0	102.3	102.4	102.5	114	147	132	132	126	98.1	99.2	2477
Mean	Nude	98.7	100.4	100.7	100.8	101.2	100	131	125	122	124	97.6	98.7	1851
	Clothed	98.7	101.5	102.2			111	143	135			97.9		

* Unable to continue after 2.4 hours—final data: 103.2, 150, 100.4 and 1589.
† Unable to continue after 2.7 hours—final data: 102.4, 156, 99.6 and 2063.

TABLE IV

Physiologic effects of wearing standard herringbone twill clothing on acclimatized men working in the heat

D.B. 93° F—W.B. 92° F

Name	Clothing	Rectal temperature °F					Pulse rate per min.					Skin temp. (Avg. wtd.) °F		Weight loss (Sweat)
				hours					*hours*				*hours*	
		0	1	2	3	4	0	1	2	3	4	0	4	*grams per hour*
Di	Nude	98.8	100.0	100.4	100.6	100.6	93	129	114	123	117	95.3	96.4	1706
	Clothed	98.5	101.0	101.1	101.3	101.4	93	135	135	123	132	95.6	97.8	1760
Kn	Nude	98.4	99.8	100.1	100.4	100.3	90	114	117	117	120	94.8	97.0	1848
	Clothed	98.5	100.9	101.2	101.0	101.0	108	120	129	129	123	95.8	98.2	1824
Li	Nude	99.7	100.7	100.6	100.7	100.6	93	117	108	117	126	96.4	96.5	1908
	Clothed	99.4	101.7	102.3	102.0	102.1	105	135	126	144	144	96.6	98.1	2353
Ma	Nude	98.4	100.3	100.7	100.8	100.6	120	150	153	141	138	95.3	96.7	1791
	Clothed	98.1	100.9	101.6	101.3	101.6	102	141	144	150	156	95.9	97.6	2244
Sz	Nude	98.0	99.4	99.5	99.8	99.8	87	108	120	123	123	95.2	96.8	1705
	Clothed	98.7	100.6	101.5	101.0	100.7	105	129	141	132	123	95.9	97.9	1760
Mean	Nude	98.7	100.0	100.3	100.5	100.4	97	124	122	124	125	95.4	96.7	1792
	Clothed	98.6	101.0	101.5	101.3	101.4	103	132	135	136	136	96.0	97.9	1988

ied. The first hour was chosen for presentation since it was the only work load accomplished by the entire group on all days. The difference in pulse rate and rectal temperature remained constant between the two groups in response to this constant work stimulus (Figure 1). The relative effect of the clothing appeared to be no greater in the unacclimatized individual than in the acclimatized.

UPPER LIMIT STUDIES

Data on the upper limits for the subjects working both nude and clothed in ordinary herringbone twill are presented in Tables II to V, and Figures

TABLE V

Physiologic effects of wearing standard herringbone twill clothing on acclimatized men working in the heat

D.B. 95° F—W.B. 94° F

Name	Clothing	Rectal temperature °F					Pulse rate per min.					Skin temp. (Avg. wtd.) °F		Weight loss (Sweat)
				hours					*hours*				*hours*	
		0	1	2	3	4	0	1	2	3	4	0	4	*grams per hour*
Hi	Nude	98.7	100.4	101.3	101.1	101.1	96	114	132	132	123	95.4	96.8	1806
	Clothed	99.0	101.7	102.8	*		114	144	135	*		96.6	*	*
Ka	Nude	98.4	100.0	100.8	100.7	101.0	114	141	147	135	156	95.6	97.2	1600
	Clothed	98.6	101.3	102.7	†		114	159	171	†		96.1	†	†
Lo	Nude	99.1	100.8	101.6	102.2	102.6	108	132	135	138	141	96.4	98.1	1771
	Clothed	99.1	102.2	103.5	‡		114	156	156	‡		96.7	‡	‡
Mi	Nude	100.0	100.1	101.0	101.0	101.4	102	129	132	138	144	95.5	97.4	2500
	Clothed	98.6	101.6	§			120	162	§			96.4	§	§
Sc	Nude	99.1	100.4	101.1	101.7	101.5	105	138	144	138	132	94.7	97.2	3087
	Clothed	98.8	101.7	102.6	103.0	102.9	108	150	153	150	156	95.9	99.2	2586
Mean	Nude	99.1	100.3	101.2	101.3	101.5	105	131	138	136	139	95.5	97.3	2153
	Clothed	98.8	101.7				114	154				96.3		

* Unable to continue after 2.2 hours—final data: 102.7, 150, 98.4 and 1804.
† Unable to continue after 2.0 hours—final data: 102.7, 171, 98.3 and 2243.
‡ Unable to continue after 2.8 hours—final data: 103.4, 150, 99.1 and 1626.
§ Unable to continue after 1.8 hours—final data: 102.9, 144, 98.3 and 2356.
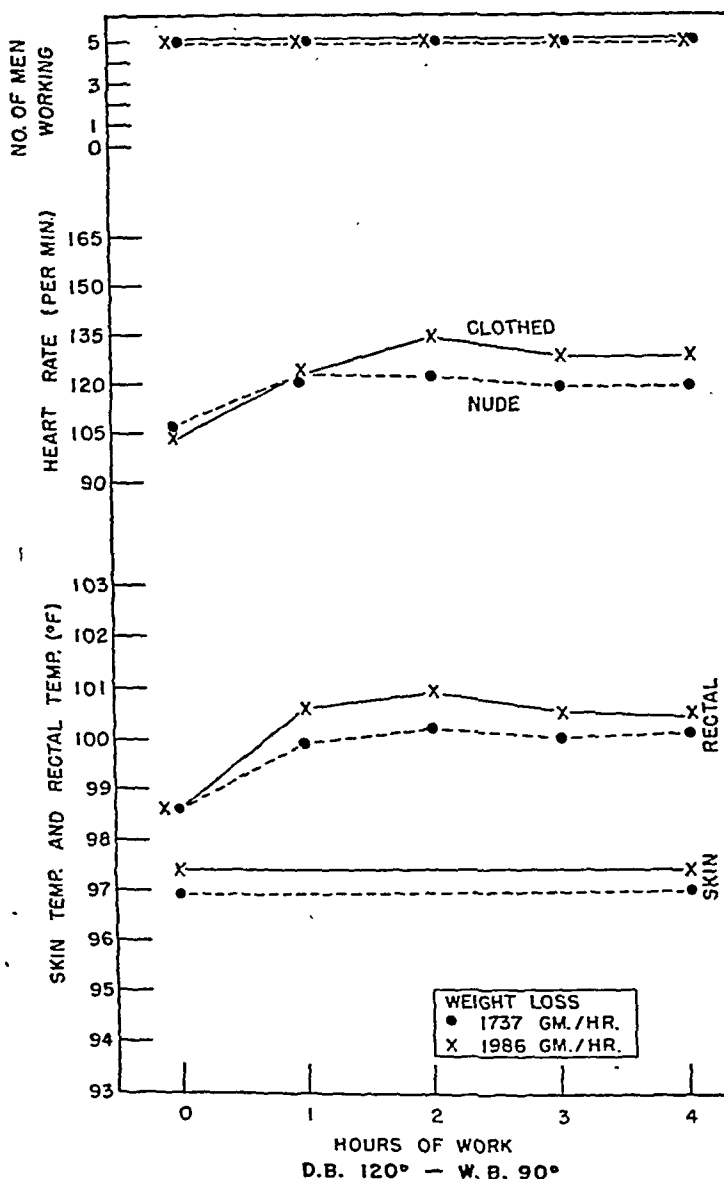
FIG. 2. AVERAGE PHYSIOLOGIC EFFECTS OF WEARING STANDARD HERRINGBONE TWILL UNIFORMS ON ACCLIMATIZED MEN MARCHING 3 MPH. AT D.B. 120°—W.B. 90° F.

This is one of the upper limiting environments for four hours of work in the clothed man. Key, ·——· nude; x——x clothed.

2 to 6. In view of the impossibility of elaborately characterizing the special impregnated garments at this time, data on the men wearing these have been omitted but all the pertinent conclusions will be presented in the text.

Environments with D.B. 120° F

When nude, subjects were able to complete the required 4 hours of marching in environments with wet bulb temperatures up to and including 92° F. However, when clothed in herringbone twill, they were able to complete, as a group, 4 hours of marching at a W.B. of 90° F. but not 92° F. At W.B. 92° F., although three of the men could finish 4 hours two were unable to complete even 3 hours of work. When clothed in the special impregnated herringbone twill the men could not complete, as a group, four hours of marching at
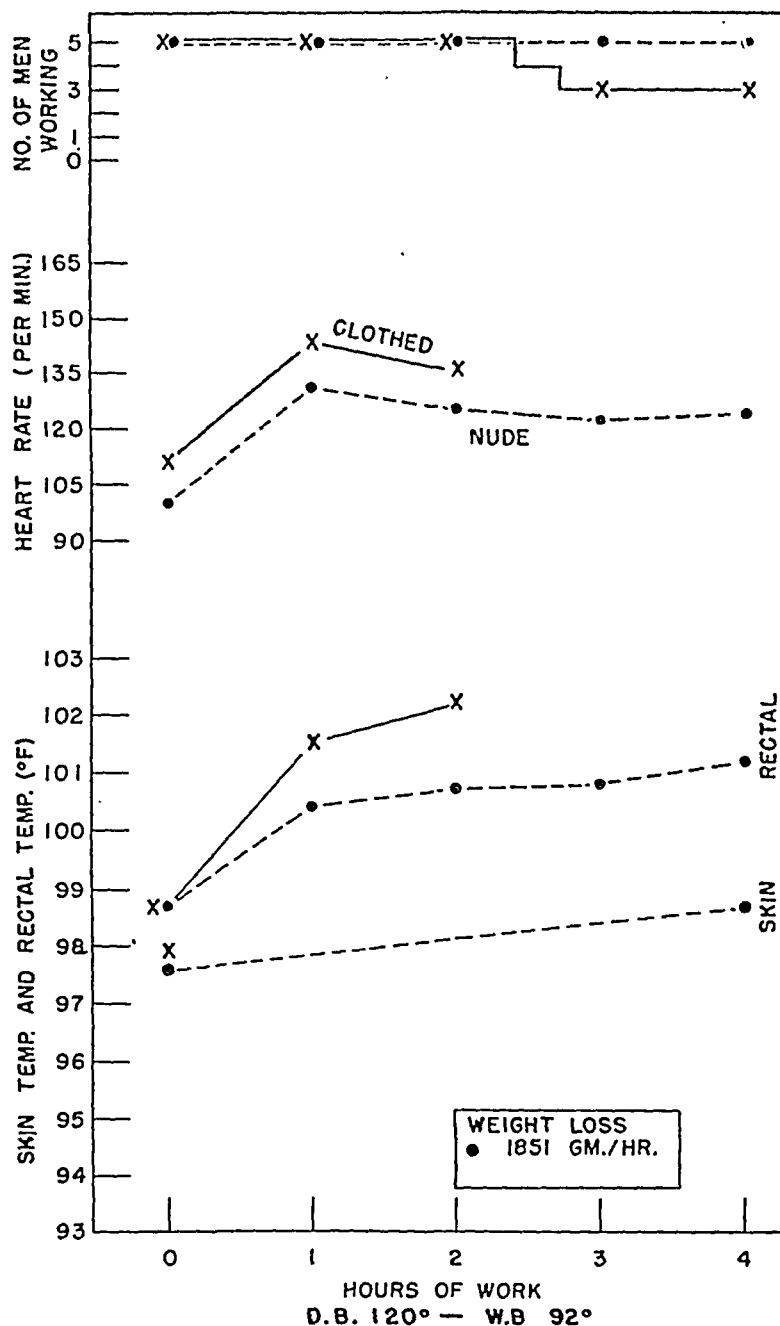
FIG. 3. AVERAGE PHYSIOLOGIC EFFECTS OF WEARING STANDARD HERRINGBONE TWILL UNIFORMS ON ACCLIMATIZED MEN MARCHING 3 MPH. AT D.B. 120°—W.B. 92° F.

This is above the upper limits for four hours of work for the group when clothed since two men failed to finish. Unless group finishes comparable period of work data not plotted. Key, ·——· nude; x——x clothed.

wet bulb temperatures above 88° F. Hence it was found (Figure 6) that the upper limiting wet bulb temperature for successful group performance of 4 hours of marching at D.B. 120° F., was 92° F. for nude men, 90° F. for herringbone twill clothed, and 88° F. for men wearing the specially treated herringbone twill uniform.

Environments with D.B. 93° and 95° F.

At D.B. 95° F., the group when nude was able to march 4 hours at wet bulb temperatures up to and including 94° F. This demonstrates the importance of the wet bulb temperature in this zone of evaporative heat regulation, in critically limiting man's performance since a reduction of the dry
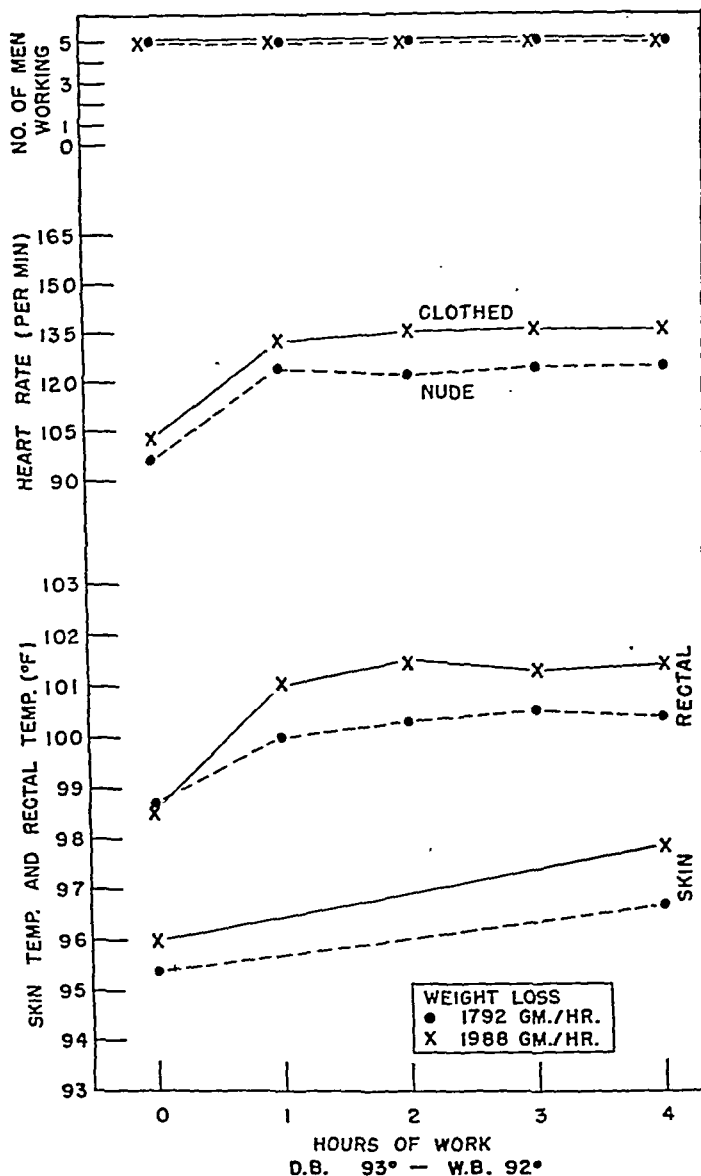
FIG. 4. AVERAGE PHYSIOLOGIC EFFECTS OF WEARING STANDARD HERRINGBONE TWILL UNIFORMS ON ACCLIMATIZED MEN MARCHING 3 MPH. AT D.B. 93°—W.B. 92° F.

This is one of the upper limiting environments for four hours of work in the clothed man. Key, ——— nude; x——x clothed.

bulb temperature by 25° resulted in elevating the wet bulb upper limit by only 2° F. When the men worked in either the untreated or treated herringbone twill, the upper limit was D.B. 93°—W.B. 92° F. Thus, in the saturated environment, the two types of clothing could not be clearly distinguished on the basis of group performance.

Physiological Responses

The effect of clothing is not restricted to its effect on work performance alone, but it is reflected also in significant physiologic changes in the rectal and skin temperatures and pulse of men working in hot climates. These physiological changes (Tables II toV) should be contrasted with the minimal effect of clothing on these men performing the standard work in a relatively cool environment. The following data compare the group averages of the nude and clothed men at the
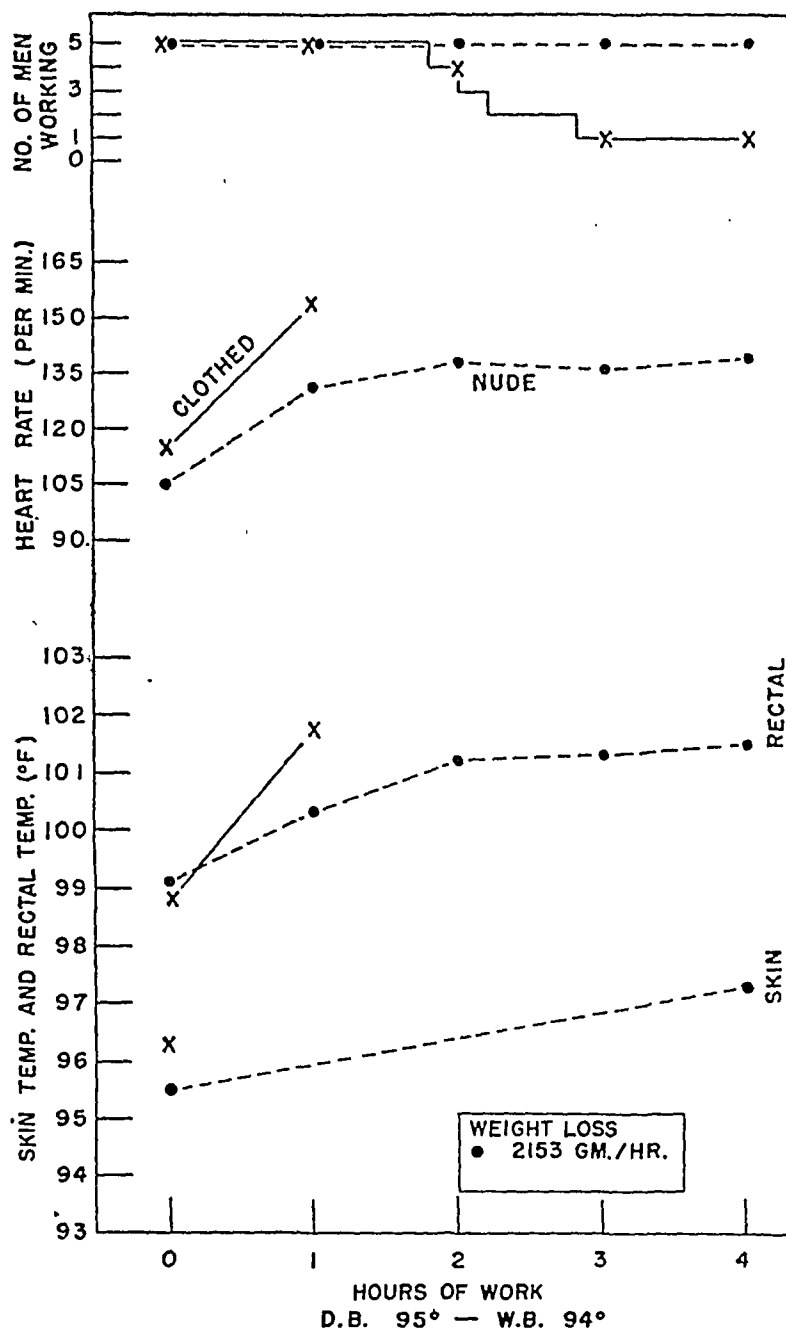
FIG. 5. AVERAGE PHYSIOLOGIC EFFECTS OF WEARING STANDARD
HERRINGBONE TWILL UNIFORMS ON ACCLIMATIZED MEN MARCH-
ING 3 MPH. AT D.B. 95°—W.B. 93° F.

This is above the upper limits for four hours of work for the
group when clothed since four of the five men failed to finish.
Unless group finishes comparable period of work data not plotted.
Key, ·————· nude; x————x clothed.

end of the four hours of marching at D.B. 77°—
W.B. 62° F.

	Rectal temp. °F	Heart rate per min.	Skin temp. °F	Weight loss grams per hour
Nude	99.6	91	90.8	143
Clothed	99.8	106	91.3	281

Clothing imposed a significant heat load in the
hot environments studied. At the end of four
hours in the two environments in which all men
finished, whether nude or clothed (Tables II and
IV) the rectal temperature of the clothed men
ranged from 0.1° to 1.5° F. higher than the same
man nude; the skin temperature of the clothed man
from 0.0° to 1.6° F. higher than nude; the pulse
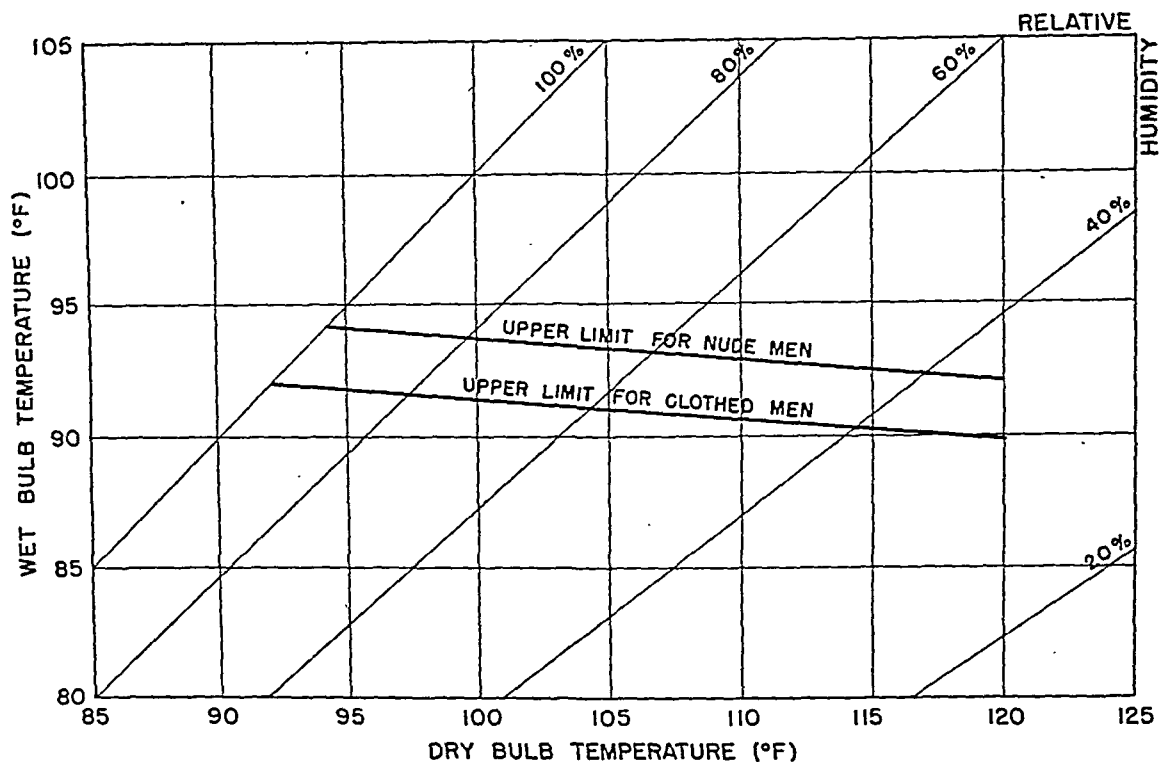
FIG. 6. EFFECT OF WEARING STANDARD HERRINGBONE TWILL UNIFORMS ON THE UPPER LIMITS OF HEAT TOLERATED BY WORKING ACCLIMATIZED MEN

The group nude or clothed was unable to work at a rate of 250 Cal. per hour for four hours at levels of heat above those shown.

rate from 3 beats lower to 18 beats higher than the nude; the sweat rate from 24 grams lower to 454 grams higher. The wearing of the special impregnated clothing resulted in proportionately greater changes which ranged up to approximately twice as great as those induced by wearing ordinary herringbone twill.

In addition to the physiologic cost, the men exhibited striking subjective reactions. These responses correlated well with the physiological findings. The men felt much cooler in the nude than when they wore either the untreated or treated clothing. In addition, the treated clothing was considered to be appreciably warmer than ordinary herringbone twill uniforms.

Weighing the clothing before and after each wearing revealed the herringbone twill clothing had absorbed approximately 1400 grams of sweat, the special clothing absorbed only half as much as this.

DISCUSSION

This study has described the acute physiological heat tolerance of a group of clothed acclimatized men exposed to several controlled external and internal heat loads. It confirms and complements observations made previously at this laboratory (6) on the upper limits tolerated by nude men. It must not be expected that industrial workers can work effectively at these levels. Rather do these upper limits indicate the maximal performance that is physiologically attainable by a group in severe heat. They may serve, therefore, as a rough guide of endurance of men in industrial operation in which optimal conditions cannot be achieved.

The specific type of work and environment, the acute nature of the experiment, the high state of acclimatization, motivation, and physical fitness of the subjects, all limit extensive direct application of this and other laboratory data. For example, the presence of a large source of radiant heat would considerably lower the limiting wet bulb temperature (10). As Von Schlictegroll (11) has stressed, ideally, specific practical studies should be carried out in the hot industries.

It is important to stress the great individual variability seen in the data. From a practical standpoint the reactions are analyzed in terms of

the average group response. However, any individual's performance and reactions may depart significantly from that of the group.

In additional work on another problem (12) at this laboratory, twelve men clothed in herringbone twill were fully acclimatized to marching at D.B. 120°—W.B. 93° and D.B. 120°—W.B. 90° F. It was found that D.B. 120°—W.B. 93° F. was above the upper limits while all the men were able to work for four hours (250 Cal. per hour) at D.B. 120°—W.B. 90° F. This finding confirmed one of the upper limits described in the present paper.

Robinson, Turrell and Gerking (8) approached the problem of limiting hot environments from the standpoint of thermal equilibrium while this laboratory has used different criteria; nevertheless, both agree as to the upper limits for the nude men. At equivalent work rate (130 Cal. per M^2 per hour) Robinson found the limiting environments for nude men similar to those reported by this laboratory, i.e. D.B. 95°—W.B. 94°; D.B. 122.0°—W.B. 92.0° F. In clothed men the limits determined by Robinson and by this study are similar for the saturated environments, but not for the 120° F. environments. Robinson's group, studying windbreak poplin, set the limit at about D.B. 122° —W.B. 86° F., while this study indicated the limit to be D.B. 120°—W.B. 90° F. for men clothed in herringbone twill and D.B. 120°—W.B. 88° F. for men in impregnated herringbone twill.

The explanation for the difference at the 120° F. environment is felt to be related to the type of clothing worn by the subjects. In these environments clothing plays a decisive rôle in determining upper limits, since evaporation and its cooling effect is limited by the type of clothing. On the other hand, in hot saturated environments evaporation of sweat is critically limited by the humidity, and the type of clothing plays a less important rôle.

SUMMARY

The effect of wearing two types of clothing on the ability of acclimatized men to work at the upper limits of heat has been evaluated in laboratory studies. The upper limiting wet bulb temperature for successful group performance of 4 hours of marching at 3 mph (250 Cal. per hour) in an environment with D.B. 120° F., was 92° F. for nude men, 90° F. for herringbone twill clothed and

88° F. for men wearing an impregnated herringbone twill uniform. The upper limiting wet bulb temperature at a D.B. 93° to 95°, was 94° F. for nude men and 92° F. for men clothed either in treated or untreated herringbone twill uniforms.

At the upper limits of environmental heat, the wearing of a single layer herringbone twill (8 oz.) uniform imposes a heat load equivalent to a 2° F. increase in the wet bulb temperature.

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AN OBJECTIVE METHOD FOR DETERMINING CIRCULATION TIME FROM PULMONARY TO SYSTEMIC CAPILLARIES BY THE USE OF THE OXIMETER

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Since the early work of Blumgart, Yens and Weiss (1 to 4) many methods have been recommended for measuring the circulation time in various segments of the circulation. In general, these methods have consisted of injecting a substance into an antecubital vein and noting its arrival either at the lung, as with the ether (5) and paraldehyde (6) tests, or at some point along the systemic circulation such as the tongue, brain or carotid body. The fact that the arm to right heart circulation time varies so widely (2, 4) even in the normal individual constitutes a disadvantage to any test which involves the injection of a substance into the antecubital veins. Moreover, subtraction of the arm to lung time (ether, paraldehyde methods) from arm to tongue or carotid body time can give only an indirect value for the measure of the pulmonary to systemic capillary time. Another disadvantage common to many of the methods described in the literature is that the end-point is either subjective or at best not very sharp. Furthermore the end-points with some of the methods are quite unpleasant and at times alarming to the patient. The CO_2 inhalation method described by Bornstein (7) in 1912 and improved by Gubner *et al.* (8) obviates intravenous injection and is a direct measure of pulmonary to systemic capillary time, but the fact that 2 breaths of 50 per cent CO_2 are necessary raises the question as to the correct starting time for the measurement. Furthermore, the end-point which consists of stimulation of respiration and flushing of the face is not always sharp. If concentrations other than 50 per cent are used, different values for the circulation time are obtained.

In the course of employing the Millikan (9) oximeter to study the degree of arterial unsaturation during breath holding, the idea presented itself that a single deep inspiration of 100 per cent nitrogen might cause a sharp drop in arterial oxygen saturation and thus serve as a measure of lung to ear circulation time. Preliminary experiments justified this assumption.

The method presented in this paper measures the circulation time from the pulmonary to systemic capillaries (lung to ear) directly, by measuring the time elapsed between a single deep inspiration of 100 per cent nitrogen and the arrival at the ear of unsaturated arterial blood. The latter is noted by use of the oximeter which gives an objective end-point independent of the interpretation of the observer or subject. It was hoped that this method would afford a truer measure of the pulmonary to systemic capillary circulation segment which is the one most frequently involved in cardiovascular disease.

EXPERIMENTAL

Subjects: Thirty-five subjects varying in age from 21 to 54, 10 of which were female, were used to obtain the normal range of values. The subjects were at rest but not under basal conditions during the observations. Three to 10 observations were made on each subject and the results averaged to obtain the circulation time. Three to 5 minutes elapsed between each trial.

Instruments: A 9 liter spirometer from a basal metabolism machine served as the reservoir of nitrogen. This was connected by 13/16" rubber tubing to a large caliber (13/16") three-way valve. The valve was fitted with a rubber mouth-piece on another arm and the third arm was in communication with room air.

The Millikan oximeter is an instrument which measures continuously the oxygen saturation of arterial blood by means of bichromatic photoelectric colorimetry of the intact fully flushed ear (9).

Procedure: Subjects were in a supine position during the period of observation. The ear unit of the oximeter was placed on the ear and the galvanometer adjusted (9). One observer manipulated the three-way valve and another watched the bell of the spirometer and the galvanometer. A nose clip was placed on the subject and he was requested to breathe room air as naturally as possible through the three-way valve. As soon as the gal-

vanometer had stabilized (approximately 30 seconds), the subject was instructed to exhale forcibly after a *normal* inspiration. *With the least possible delay* after completion of the forced expiration, the valve was turned connecting the subject to the nitrogen reservoir and he was instructed to inhale as quickly and as deeply as he could. At the end of the forced inspiration, the valve was again turned to room air and the subject asked to resume normal respiration. Two or three practice trials generally sufficed to obtain the cooperation of the subject. One observer registered, by means of a stopwatch, the beginning of inspiration as signalled by descent of the spirometer and the *start* of the fall in galvanometer reading. The interval between the beginning of inspiration and the beginning of the downward deflection of the galvanometer was considered to be the circulation time. The full galvanometer deflection was noted and the test repeated when the beam had returned to its original position.

In carrying out this technique, it is important that the subject inhale quickly after maximum expiration, since prolongation of the expiratory pause will cause sufficient arterial desaturation to deflect the galvanometer and give an abnormally rapid circulation time. It is also important that the subject be requested to inspire as rapidly as possible in order to decrease the duration of inspiration to a minimum.

RESULTS

A deep breath of 100 per cent nitrogen was found to be innocuous in normal individuals and the subjects were not aware of the fact that they were breathing 100 per cent nitrogen. Most of the subjects noted a slight diminution in light perception and/or dizziness coming on several seconds after the inhalation of nitrogen and lasting for a few seconds. Electrocardiographic tracings were recorded on some of the subjects. There was a slight increase in rate during the forced expiration and a slowing immediately after the deep in-

TABLE I

Pulmonary to systemic capillary circulation times in 35 normal subjects

Subject	Sex	Age	Height	Weight	Circulation time	Galv. defl.	Vol. N₂	Pulse after inhalation N₂	
								Immediately	One min. later
		years	*inches*	*pounds*	*seconds*	*mm.*	*ml.*	*per min.*	*per min.*
S.H. 1	Female	27	60	115	4.9*	10*			
A.W. 9	Female	31	60	112	5.2	6	2220*	74*	70*
G.M. 10	Female	21	68	148	4.9	12	3770	95	68
S.F. 11	Female	30	62	125	5.2	14	2970	.88	68
L.P. 13	Female	23	62	118	5.4	15	2580	76	64
D.W. 14	Female	30	65	130	5.2	11	2710	104	88
A.K. 15	Female	29	62	110	4.8	11	2560	86	70
R.F. 16	Female	22	67	140	4.7	10	3560	108	86
P.D. 19	Female	25	65	143	4.1	10	2140	96	88
H.K. 23	Female	24	60	99	4.3	12	3440	118	104
H.T. 2	Male	30	71	185	5.3	17	4140		
L.B. 3	Male	30	72	182	6.8	11	5110		
A.G. 4	Male	37	69	160	6.2	7	3260	100	88
R.H. 5	Male	35	70	165	5.8	14	4570	91	82
J.W. 6	Male	31	73	180	5.6	13	4350		
B.M. 7	Male	30	73	210	7.0	12	3480	68	68
B.K. 8	Male	28	69	145	4.8	11	3150	86	74
J.W. 12	Male	29	74	175	5.1	16	4140	88	76
K.V. 17	Male	28	69	150	5.3	11	3570	78	68
M.C. 18	Male	28	71	180	5.0	15	5150	90	82
C.B. 20	Male	26	71	145	5.8	14	4460	84	78
J.T. 21	Male	32	66	148	6.5	17	3530	80	70
C.C. 22	Male	33	67	170	5.7	18	2770	84	68
W.M. 24	Male	27	68	165	5.0	15	3580	102	92
O.B. 25	Male	44	66	181	5.3	12	2690	92	88
F.C. 26	Male	36	68	160	6.9	16	4300	64	58
P.D. 27	Male	34	72	199	5.4	14	4670	92	76
J.E. 28	Male	27	69	200	4.9	14	4000	80	66
I.F. 29	Male	33	68	163	5.2	12	3460	78	64
J.W. 30	Male	32	69	160	4.8	13	4080	96	80
A.D. 31	Male	32	66	168	5.3	15	3990	102	88
L.W. 32	Male	31	67	145	4.9	11	3150	94	86
C.C. 33	Male	38	71	186	4.4	13	4010	98	82
R.H. 34	Male	33	68	142	5.1	11	3290	86	76
H.T. 35	Male	54	65	130	5.4	6	2260	104	100

* Each figure in these columns represents an average of 3 to 10 observations.

spiration which lasted for about 10 seconds. These changes were missed when the pulse was recorded manually and only a slight increase in rate was noted during the first minute after the deep inspiration of nitrogen.

The results are presented in Table I and Figure 1. The circulation time for the 35 normal subjects ranged between 4.1 and 7.0 seconds with an average value of 5.2 seconds. The values for 23 or 66 per cent of the subjects were in the range of 4.6 to 5.5 seconds (Figure 1). The values for the circulation time were reproducible within a very small range in any one individual. The measurements obtained by repeated trials (3 to 10) did not differ from each other by more than 0.1 second in the most consistent individual, and by not more than 1.8 in the most variable individual. As can be seen from Table I, there was considerable variation in the average volume of nitrogen inspired by the 35 subjects, but this did not influence the sharpness of the end point or the circulation time. The average volume of nitrogen inspired per trial by 34 subjects was 3560 ml. and the average galvanometer deflection was 12.5 mm. for the 35 subjects.

DISCUSSION

It has been shown that in normal subjects, a single inspiration of nitrogen will cause a fall in arterial blood oxygen saturation which can be recorded by the oximeter. This fact has been utilized in determining the circulation time from lung to ear. In 35 subjects without heart disease the range of values was found to be 4.1 to 7.0 seconds. These values are probably higher than the true pulmonary to systemic capillary circulation time since the measurement actually includes the time of inspiration, diffusion time of the nitrogen in the residual air, the galvanometer lag and the reaction time of the observer.

Most of the methods described for determining the blood velocity in this segment of the circulation have employed two separate observations, a determination of the arm to lung circulation time by use of paraldehyde (6) or ether (5) and arm to tongue (10 to 14) or carotid body (4). The pulmonary to systemic capillary circulation time is then calculated by subtracting the former from the latter. Since this segment of the circulation
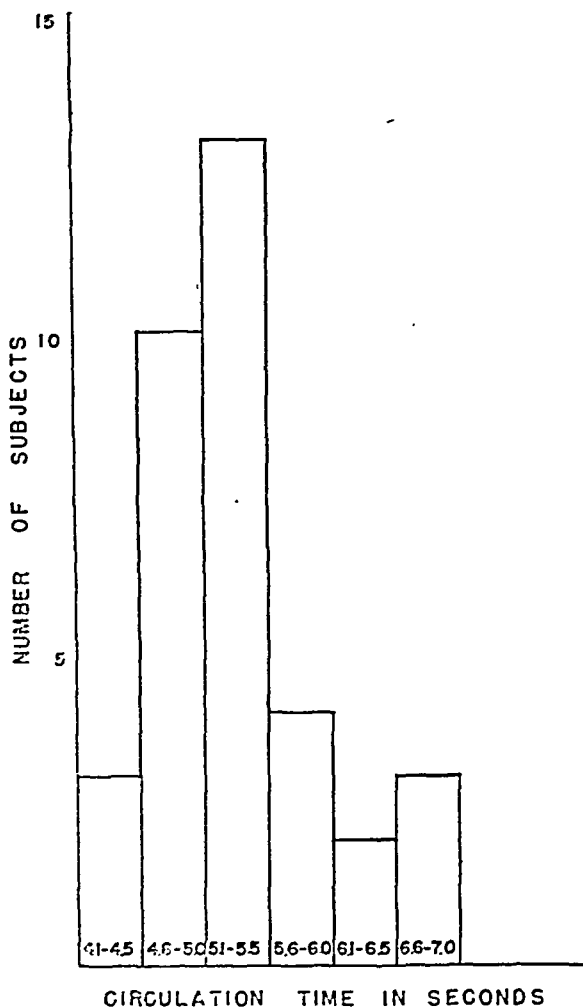
FIG. 1. CIRCULATION TIME FROM PULMONARY TO SYSTEMIC CAPILLARIES BY THE USE OF THE OXIMETER
Distribution of values obtained in 35 normal subjects.

is less variable in normals (2, 4), and since it is the segment most commonly affected in heart disease (failure of left ventricle), a direct method to measure the pulmonary to systemic capillary circulation time may have a special field of usefulness. The values obtained by Gubner *et al.* with the CO_2 inhalation method (5 to 10 seconds) are higher and have a wider range than our normal values. This difference may be partially explained by the somewhat less precise end-point (stimulation of respiration and flushing of face) and the fact that two breaths of CO_2 were taken and timing started from the first inspiration whereas the stimulation of respiration may have been the result of the second breath.

The present observations suggest also that inhalation of 100 per cent oxygen might be used to measure the circulation time. Such a use might be of advantage because of the greater availability of 100 per cent oxygen, and would be particularly advantageous in patients with arterial unsaturation. However, in subjects with normal arterial saturation, the magnitude of the galvanometer deflection resulting from one deep breath of 100 per cent oxygen is too small for accurate results. It is probable that in subjects with decreased arterial saturation, inhalation of 100 per cent oxygen would give greater galvanometer deflections which would give greater assurance that a change in the reading was not due to chance. In this connection it should be pointed out that the galvanometer setting of the oximeter is arbitrary. In normal subjects, the setting is usually made to correspond to a 95 to 98 per cent arterial saturation. In patients with arterial unsaturation, the galvanometer setting would arbitrarily have to be made at 80 to 90; an increase in saturation resulting from a breath of pure oxygen should manifest itself by a sizeable increase in the galvanometer reading.

SUMMARY

1. A new objective method for measuring pulmonary to systemic capillary circulation time is described. It is based on the observation that a single deep breath of 100 per cent nitrogen causes a decrease in arterial oxygen saturation which can be recorded by the oximeter.

2. The advantages of this method are its objectivity, simplicity and the avoidance of the widely variable arm to heart segment of the circulation. It offers a direct procedure to test the efficiency of the left side of the heart.

3. The range in 35 normal subjects was found to be 4.1 to 7.0 seconds with an average of 5.2 seconds.

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CLINICAL USES OF 2,3–DIMERCAPTOPROPANOL (BAL). I. THE SYSTEMIC TREATMENT OF EXPERIMENTAL ARSENIC POISONING (MAPHARSEN, LEWISITE, PHENYL ARSENOXIDE) WITH BAL [1]

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INTRODUCTION

There is now a convincing body of evidence that the toxic effects of arsenicals are referable primarily to the fact that they combine with -SH groups in the tissues, and thus block one or more physiologic sytems vital to the cellular economy. The toxic action of 3-amino-4-hydroxyphenyl arsenoxide against both trypanosomes and rats has been shown to be diminished or abolished by cyseine or glutathione (1 to 3). Cysteine also delayed the toxic action of sodium arsenite in mice (4); and a similar inhibition of the neurotoxic action of sodium arsenite was observed *in vitro* (5). It was further shown (6, 7, 7a) that the toxic effects of arsenicals were not only prevented, but could actually be reversed by -SH compounds. Protozoa (*Colpidium*) already immobilized by diphenyldichloroarsine, and spirochetes (*S. pallida*) immobilized by phenyl arsenoxides, were resuscitated on the addition of monothioethyleneglycol and cysteine respectively.

The foregoing observations were indicative of the strong affinity of arsenicals for -SH groups, as already noted by Ehrlich (8), but did not necessarily prove that arsenicals owed their toxic effects to the fact that they combined with -SH groups in tissue. The missing link in this chain of evidence was provided by the observation that when arsenicals combined with tissue proteins, the reactive -SH groups of the latter simultaneously disappeared (9 to 11). The widely varying systemic toxicity of a series of phenyl arsenoxides was shown to be correlated with, and probably determined by, the varying degree to which they were bound by the host tissues (12); and a high degree of correlation was noted (13) between the trypanocidal action of phenyl arsenoxides and the extent to which they were bound by the trypanosomes. Finally, a series of enzyme proteins containing free -SH groups were shown to be reversibly inactivated by arsenicals *in vitro*, with the disappearance of the titratable -SH groups (14, 15). The implication that the toxic action of arsenicals is referable to the inactivation of similar -SH-containing enzyme proteins in living cells is clear.

Although monothiol compounds had been found to protect against the toxic action of some arsenicals, and in isolated cases actually to reverse that toxic effect, the antidotal action was not regular. Cysteine and glutathione delayed, but did not prevent, the toxic action of sodium arsenite on the medullated nerves of frogs, measured either by their respiration or by their action potential (5). Moreover, once the action current had been abolished, even a large excess of sulfhydryl compound failed to induce recovery. Similarly, monothiols in general failed to prevent the inhibition by sodium arsenite or Lewisite of the pyruvate oxidase system in a pigeon brain "brei" (15, 16), or the respiration of rat skin slices (16), and failed also to protect human or rat skin from the vesicant action of Lewisite (17, 18). Even with the trivalent aromatic arsenicals studied by Voegtlin and his co-workers and by Eagle, a 10- to 40-fold excess of cysteine or glutathione was required to prevent toxic effects *in vitro;* the latter compound was most effective *in vivo* if administered immediately before the arsenical (2); and if even a few minutes were allowed to elapse between the injection into rabbits of a lethal dose of the 3-NH₂-4-OH-phenyl arsenoxide and a following injection of cysteine or glutathione, the latter had no protective action (Table I).

TABLE I

The protection of rabbits against an acute lethal dose of mapharsen by a single intravenous injection of BAL, and the absence of such protection with cysteine or glutathione

-SH compound used to detoxify mapharsen		Outcome		Protective dose of -SH compound		
		Dead	Survived	PD-₉₅	PD₅₀	
Cysteine	*mgm. per kgm.* 800 400 200	2 6 4	0 0 0	*mgm. per kgm.* >800	*mgm. per kgm.* >800	No protection even with doses approaching the lethal level, and representing a 15- to 60-fold molar excess relative to the arsenical.
Glutathione	400 300 200	2 1 3	0 0 0	>400	>400	
BAL	36 24 12 6 2.4	0 0 1 2 2	4 3 2 1 1	25 (0.20 millimols per kgm.)	7.5 (0.06 millimols per kgm.)	A lethal dose of mapharsen was neutralized *in vivo* by 1 to 3 molar equivalents of BAL.
0 (Controls: No -SH compound)*		0	5	0		

Rabbits received 20 mgm. per kgm. (0.008 millimols per kgm.) of mapharsen intravenously (LD₅₀ = 13 mgm. per kgm.; LD>₉₅ = 17 mgm. per kgm.). Five minutes later, the indicated amount of BAL (NDR133-11), cysteine or glutathione in 0.85 per cent NaCl at pH 7.4 was also injected intravenously. The LD₅₀ value of this particular lot of BAL on intravenous injection in rabbits was 40 to 50 mgm. per kgm.

* Control animals, receiving no -SH compound, died in 30 to 60 minutes. In animals receiving cysteine and glutathione, death was not significantly delayed. In the case of animals receiving BAL, those listed as "survived" were alive and well 30 days after treatment. In most of those which died despite BAL, death was significantly delayed, the time varying between 20 minutes and 8 days after treatment.

An important and fruitful advance was made with the discovery (11) that when Lewisite reacted with keratin, approximately 75 per cent of the bound arsenic was in combination with two thiol groups. This suggested the formation of a relatively stable ring structure such as

$$R—As\begin{array}{c} S—C< \\ | \\ S—C< \end{array};$$
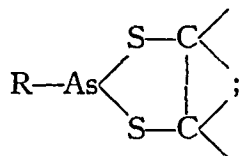
and it seemed possible that "the high toxicity of trivalent arsenicals might be due to their ability to combine with essential -SH groups in certain tissue proteins to form stable arsenical rings" (15). Conversely, in a search for compounds capable of acting as decontaminants or antidotes for toxic arsenicals, Stocken and Thompson reasoned that "simple dithiol compounds might form relatively stable ring compounds with Lewisite or other trivalent arsenicals, and might consequently compete effectively with the dithiol proteins in the tissues" (15). Accordingly, a large series of dithiol compounds was prepared and tested with respect to (a) reactivity with arsenicals and the stability of the formed thioarsenites (16), (b) ability to protect enzyme systems from the toxic effects of Lewisite and other toxic arsenicals (17, 18), and (c) protective action on local application to a skin area contaminated with vesicant or even lethal amounts of Lewisite (18, 19). These extensive studies, summarized in part in two recent reviews (20, 21) brilliantly confirmed the original thesis. The cyclic thioarsenites formed by the interaction of simple dithiols and trivalent arsenicals proved far more stable than those formed by monothiols or by the interaction of tissue proteins and dithiols; and presumably in consequence of that stable ring structure, these dithiols could successfully compete with tissue proteins for such toxic arsenicals as Lewisite or phenyldichloroarsine. Enzyme systems were protected *in vitro* even 1 hour after the introduction of an arsenical vesicant; and local decontamination of the skin or eye with a dithiol prevented or minimized the appearance of local toxic manifestations.

Of the numerous dithiol compounds prepared and tested by Stocken and Thompson and their co-workers, one in particular, the 2,3-dimercaptopropanol,

$$CH_2CHCH_2OH$$
$$\quad | \quad | \qquad ,$$
$$\quad SH \ SH$$
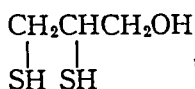
recommended itself as a local decontaminant in combating the arsenical blister gases. This compound, generally known as "BAL" (British anti-Lewisite) was compounded in ointment form for use on the skin and eyes, and the value of such preparations was abundantly demonstrated in numerous experimental studies.

THE EFFICACY OF BAL (IN AQUEOUS OR PROPYLENE GLYCOL SOLUTION) IN THE SYSTEMIC TREATMENT OF EXPERIMENTAL ARSENIC POISONING

The early use of BAL was limited to its local application in the treatment of experimental Lewisite burns, or of the arsenical dermatitis resulting from antisyphilitic treatment. It seemed clear, however, that the drug so applied had a systemic as well as local action. Thus the application of BAL to a skin area of rats 15 minutes after its contamination with Lewisite resulted in a 4-fold increase in the urinary excretion of arsenic (22). Similarly, the urinary excretion of arsenic increased in human cases of exfoliative dermatitis caused by mapharsen, and treated by the local application of BAL ointment (23). Moreover, BAL ointment was therapeutically effective in such cases when applied to normal skin distant from the lesion. These results clearly indicated that BAL was absorbed through the skin in sufficient quantity to have a systemic effect; and it seemed likely that BAL given by injection might have a therapeutic effect in the treatment of arsenic poisoning.

This was borne out by the finding (19) that aqueous solutions of BAL (50 to 60 mgm. per kgm.) injected intraperitoneally into rats 1 to 2 hours after the skin application of a lethal dose of Lewisite saved 67 to 83 per cent of the animals, and that all were saved by an initial dose of 60 to 70 mgm. per kgm., followed by a second smaller dose 3½ hours later.

Our initial experiments on this point (24) dealt with animals poisoned by the systemic administration of mapharsen. As shown in Table I, rabbits injected intravenously with a single large dose of mapharsen (20 mgm. per kgm., or 0.08 millimols per kgm.), and which otherwise died in 30 to 60 minutes, were saved if treated with BAL 5 minutes after the arsenical. Approximately 25 mgm. per kgm. BAL, or 0.20 millimols per kgm., protected all the animals, and 7.5 mgm. per kgm. (0.06 millimols per kgm.) protected half. The revivifying effect in the animals, many of which were already in acute distress at the time of administration of the BAL, was striking. Under the conditions of this acute experiment, the arsenical was therefore neutralized in vivo by 1 to 3 moles of BAL. In marked contrast, neither cysteine nor glutathione, similarly injected in amounts approaching the lethal level (800 and 400 mgm. per kgm., respectively), had a demonstrable protective action. These results were qualitatively confirmed in cats (25).

A more rigorous test of the detoxifying action of BAL was provided by rabbits which had received 8 mgm. per kgm. mapharsen at hourly intervals, for a total of 4 injections. This procedure gave the arsenical time to be fixed by the tissues, and corresponded more closely to the serious treatment reactions encountered in the course of antisyphilitic treatment, as well as the systemic arsenic poisoning observed after skin burns with Lewisite. With this drastic form of cumulative arsenic poisoning, 15 per cent of the animals (34 of 223) had died before treatment with BAL was begun, and many of the remainder were moribund. The detoxifying action of BAL in these animals was not as striking as that observed after the acute injection of a single massive dose, but was none the less definite. As shown in Table II, on all the treatment schedules used, and at total BAL dosages varying between 10 and 80 mgm. per kgm., approximately 40 per cent of the animals were saved, and in an additional 35 per cent death was appreciably delayed. As shown in the right-hand section of the Table, BAL in propylene glycol administered by inunction at similar intervals, in individual doses of 10 to 80 mgm. per kgm., and total doses of 40 to 320 mgm. per kgm., also saved some of the rabbits, but was somewhat less effective in this respect than much smaller doses given by injection. Similar results were obtained with aqueous solutions of BAL, in mice poisoned

TABLE II

The efficacy of BAL (1 to 10 per cent solution in propylene glycol) in the treatment of rabbits poisoned with multiple doses of mapharsen

Frequency and number of BAL injections	Rabbits injected intravenously, subcutaneously or intramuscularly				Rabbits treated by inunction of BAL solution			
		Died		Survived		Died		Survived
		<5 days	>5 days			<5 days	>5 days	
Single injection 1 hour after last mapharsen	*mgm./kgm. at each injection* 40 20 10 5	4 3 1 2	1 3 3 5	4 3 5 2	*mgm./kgm. at each inunction* 160 80 40 20	2 1 1 1	1 0 0 1	0 2 2 1
	Totals	11	12	15		5	2	5
4 injections, at 2 hourly intervals, beginning 1 hour after last mapharsen	20 10 5 2	5 2 2 1	2 4 3 3	1 4 4 2	80 40 20 10	2 0 2 2	1 2 1 1	0 1 0 0
	Totals	10	12	11		6	5	1
4 injections, at 24-hour intervals beginning 1 hour after last mapharsen	20 10 5	3 1 1	2 5 4	4 3 4	80 40 20	0 0 1	3 2 1	0 1 2
	Totals	5	11	11		1	6	3
GRAND TOTAL on all schedules of injection, and all dosages		26 27 per cent	35 36 per cent	37 38 per cent		12 35 per cent	13 38 per cent	9 26 per cent

Mapharsen (8 mgm. per kgm.) was injected intravenously 4 times at hourly intervals. One hour after the last injection, BAL was injected at the dosage and frequency indicated in the Table. There was no significant difference between rabbits injected intravenously, intramuscularly or subcutaneously, and they are not distinguished in the Table.

with skin applications of Lewisite (26) and in dogs exposed to Lewisite vapor (27). In both groups, animals could be saved by systemic treatment which were not protected by skin application alone.

Although BAL in aqueous or propylene glycol solution given systemically was thus definitely effective in the treatment of arsenic poisoning, the practical difficulty lay in its rapid deterioration in such solutions. It seemed clear that if BAL could be compounded in a stable preparation suitable for injection, one might be able to avoid the application of an irritating ointment or solution to an already inflamed or exfoliating skin area, and to control the dosage accurately. More important, such an injectable preparation might prove effective in the treatment, not only of arsenical dermatitis, but also of the systemic arsenic poisoning which sometimes follows exposure to the arsenical blister gases, as well as the serious toxic reactions other than dermatitis often re-

sulting from the intensified treatment of early syphilis.

A STABLE SOLUTION OF BAL SUITABLE FOR SYSTEMIC ADMINISTRATION

1. Stability of BAL in peanut oil and benzyl benzoate. The development of preparations suitable for parenteral use presented difficulties chiefly because of the instability of the BAL itself. Although saturated aqueous solutions of BAL had been administered intravenously, intraperitoneally, intramuscularly and subcutaneously in experimental animals, such solutions could not be sterilized, and had to be freshly prepared, seriously limiting their usefulness. Propylene glycol was a desirable solvent in many respects, but the solutions were locally irritating, and the BAL in such solutions slowly deteriorated (28). A satisfactory vehicle was provided with the finding that BAL was soluble in peanut oil to approximately 5 per cent

(weight/volume), and that with the addition of 2 parts of benzyl benzoate for each part of BAL, it was miscible with peanut oil (*Oleum arachis*) in all proportions (24). Such solutions were homogeneous at ordinary room temperatures, became cloudy when stored at ice-box temperatures, but regained their normal appearance on warming. The solutions could be ampuled and sterilized by autoclaving at 120° C. for 20 minutes, or by oven sterilization at 160° C. for 1 hour. In our early experiments such treatment of 5 per cent solutions caused a loss of titratable sulfhydryl groups of up to 11 per cent. This was not increased by further heating, as by daily autoclaving for 20 minutes on 4 successive days, or by storage at 63° C. for 6 weeks. In lots of BAL subsequently processed in this laboratory, using different batches of peanut oil and benzyl benzoate, the loss in -SH groups on the similar sterilization of solutions in peanut oil containing 10 per cent BAL and 20 per cent benzyl benzoate varied between 0.8 to 5.5 per cent. The pH of the peanut oil-benzyl benzoate solution has been reported to be an important factor in the loss of titratable -SH groups on heating, and the minimum loss of 1.5 to 2.5 per cent was observed at pH 6.0 and 6.6 respectively (29). In our hands, however, when there was no admixture with water or alcohol, only minor differences in the degree of loss were observed in the apparent pH range 4.3 to 7.7 (Table III).

As of February 5, 1945, 102 different lots of BAL solution had been commercially processed for the armed forces. Each lot consisted of 10,000 ampules, each containing 4.6 to 4.7 ml. of a peanut oil solution of BAL (10 grams BAL and 20 grams benzyl benzoate per 100 ml. solution). The glass-sealed ampules, with an average of approximately 2 ml. air space, were sterilized at 160 to 170° C. for 1 to 1¼ hours. The percentage deterioration in these 102 lots, measured by the assay of free -SH groups, varied between 0 and 8.43 per cent, averaging 2.53 (30). No attempt was made to control the acidity of the solution, and in 8 representative lots, in which the degree of inactivation varied between 0 and 7.4 per cent, the pH of aqueous extracts was found in this laboratory to vary between 4.1 and 4.7, without apparent relation to the degree of inactivation.

This slight decomposition of BAL was probably

TABLE III

The stability of a solution of BAL in peanut oil and benzyl benzoate at varying pH

5 per cent BAL and 10 per cent benzyl benzoate in peanut oil			10 per cent BAL and 20 per cent benzyl benzoate in peanut oil		
pH of aqueous extract		Percentage loss of titratable -SH groups	pH of aqueous extract		Percentage loss of titratable -SH groups
before heating	after heating		before heating	after heating	
2.47	3.40	9.0	2.05	3.48	6.1
2.66	3.61	8.4	2.30	3.86	5.7
*4.30	4.50	5.5	4.36	4.98	4.6
6.04	5.50	3.5	6.17	6.17	3.7
6.52	5.75	3.5	6.62	6.48	4.5
6.66	6.48	4.0	7.04	6.89	4.0
7.38	7.12	3.0	7.36	7.18	6.0
7.74	7.58	4.0	7.74	7.43	9.5

HCl (or NH₃) gas passed through a peanut oil solution containing 10 grams BAL and 20 grams benzoate per 100 ml. These solutions were then mixed with the stock untreated solution in varying proportions, and each dilution was further mixed with an equal volume of peanut oil to give solutions containing 5 per cent BAL and 10 per cent benzyl benzoate. The pH of aqueous extracts of each preparation was determined (29) before and after sterilization of the oil in glass-sealed tubes under air at 170° C. (1 hour). Two ml. of each mixture were shaken vigorously with 2 ml. H₂O for 2 minutes. The H₂O layer was removed after centrifugation, and its pH determined with a glass electrode (Beckman pH meter). The degree to which the BAL had deteriorated on sterilization was determined by direct iodometric titration of a suspension of the oil in a large excess of water.

* Stock solution, not treated with HCl or NH₃.

due in part to oxidizing substances in the peanut oil, in part to the presence of traces of water, and to a lesser extent, to the small amount of oxygen in the free air space of the tube in which the solution was sterilized. Once these substances had been consumed, there was no further loss on additional heating or aging. While this degree of loss is slight, it should nevertheless be measured in the preparation and processing of every lot of BAL solution.

2. Toxicity of peanut oil-benzyl benzoate solution of BAL in rabbits. The toxicity of the BAL-benzylbenzoate-peanut oil mixture in rabbits is summarized in Tables IV and V. The toxicity was a function of the total amount of BAL administered, and was independent of its concentration. In studying the tissue damage produced by such solutions at the site of injection, 5 and 10 per cent solutions in peanut oil, containing 10 and 20 per cent respectively of benzyl benzoate, did not cause undue local damage when injected intra-

TABLE IV

*The toxicity in rabbits of a solution of BAL in peanut oil and benzyl benzoate**

Frequency of injection	Method of administration	BAL each injection	Died	Survived**	Maximal tolerated dose per injection†	LD₅₀ per injection†
		mgm. per kgm.			*mgm. per kgm.*	*mgm. per kgm.*
Single injection	Intramuscular	80 / 60 / 40	3 / 3 / 0	0 / 2 / 6	40	60
	Subcutaneous	80 / 60 / 40	3 / 1 / 0	0 / 4 / 5	40	60 to 80
Multiple injections (repeated at 4-hour intervals for total of 4 injections)	Intramuscular	40 / 30 / 25	4 / 1 / 0	1 / 4 / 5	25	35
Multiple injections (repeated at 2-hour intervals for total of 4 injections)	Intramuscular‡	40 / 30 / 25 / 20	3 / 4 / 2 / 1	0 / 1 / 3 / 4	15 to 20	25
	Subcutaneous	30 / 25 / 20	3 / 1 / 0	2 / 4 / 5	20	25 to 30

* 5 grams BAL and 10 grams benzyl benzoate up to 100 ml. with peanut oil.
** Alive and well after thirty days.
† Because of small number of animals, these values are approximations only.
‡ Followed by daily intramuscular injections at same dosage level for 6 days. This probably had only a slight effect on toxicity.

TABLE V

The effect of the concentration of BAL on its acute and cumulative toxicity on intramuscular injection into rabbits

Solution injected			Schedule of injections							
			Single injection		4 injections at 2-hour intervals		4 injections at 4-hour intervals		4 injections at 8-hour intervals	
BAL	Benzyl benzoate	Peanut oil	LD₅₀	MTD	LD₅₀	MTD	LD₅₀	MTD	LD₅₀	MTD
per cent	*per cent*									
20	40	to volume	85	70	100	75	140	100	185	140
5	10	to volume	80	60			145	110		
5	to volume	none					120			

(All doses are expressed as total mgm. BAL per kgm. For toxic levels per injection, these should be divided by 4.)

CONCLUSION: The systemic toxicity of BAL on intramuscular injection in rabbits was independent of the concentration of BAL or benzyl benzoate in the range studied. There was definite cumulative toxicity if injections were repeated at 8 hour intervals or less.

Concentrations of BAL and benzyl benzoate are expressed as grams per 100 ml. solution. LD₅₀=dose of BAL which killed half of animals, and MTD="maximal tolerated dose" (less than 10 per cent mortality), in both cases expressed as total mgm. BAL per kgm.

muscularly in dosages comparable with those recommended for use in man (31). In general, the tissue reaction to such doses at the site of injection was not appreciably greater than that produced by the intramuscular injection of the suspensions of bismuth subsalicylate in oil as used in the treatment of syphilis. BAL dissolved in benzyl benzoate alone caused somewhat greater local reaction than the solutions in peanut oil.

It should be noted in Tables IV and V that, although BAL is known to be rapidly excreted (22), there was definite cumulative toxicity when injections were repeated at short intervals. In rabbits, the maximum tolerated dose of a given

preparation on single intramuscular injection was 60 to 70 mgm. per kgm. This was decreased to 35 mgm. kgm. per injection when 4 doses were given at 8-hour intervals, and was further decreased to 28 to 20 mgm. per kgm. when the injections were repeated at 4- and 2-hour intervals respectively. In determining the optimum dosage schedule for man, it was therefore necessary to strike a balance between the desirability of prompt and intensive early treatment with BAL, and the cumulative toxicity of injections repeated at short intervals (32, 24).

THE EFFICACY OF BAL IN PEANUT OIL AND BENZYL BENZOATE IN THE SYSTEMIC TREATMENT OF EXPERIMENTAL ARSENIC POISONING

1. Mapharsen poisoning. Solutions of BAL in peanut oil injected intramuscularly were as active as the solutions in propylene glycol in protecting rabbits poisoned with repeated large doses of mapharsen (24) (Table VI). Of the various schedules tried for the administration of the BAL, one of the most effective consisted of 4 injections at 2-hour intervals, followed by single daily injections for 6 days. On that schedule, BAL in doses of 1 to 10 mgm. per kgm. per injection saved 55 per cent of the animals, and death was significantly delayed in an additional 22 per cent.

2. Lewisite and phenylarsenoxide poisoning. In rabbits injected subcutaneously with a solution of Lewisite in propylene glycol at twice the LD_{50} level, treatment with BAL in peanut oil and benzyl benzoate was effective if begun 2 hours after the injection of the arsenical (24) (Table VII). In contrast to the results obtained in

TABLE VI

The efficacy of a solution of BAL in peanut oil and benzyl benzoate in the treatment of massive arsenic poisoning in rabbits

Method of treatment with BAL solution	BAL per injection	Died		Survived*
		<5 days	>5 days	
Single injection	*mgm. per kgm.* 20 10 5 2.5	2 4 1 2	2 0 4 4	4 4 2 1
	Total	9 30 per cent	10 33 per cent	11 37 per cent
Four injections, at 2-hour intervals	10 5 2.5 1	1 1 3 2	6 5 3 4	3 4 4 4
	Total	7 18 per cent	18 45 per cent	15 37 per cent
Four injections, at 24-hour intervals	10 5 2.5	4 2 3	0 4 4	6 4 2
	Total	9 31 per cent	8 28 per cent	12 41 per cent
Four injections on first day, at 2-hour intervals, followed by single daily injections for 6 days	10 5 2.5 1	3 2 2 2	1 2 3 3	5 7 5 5
	Total	9 22.5 per cent	9 22.5 per cent	22 55 per cent
Grand Totals (All schedules of injections, all dosages)		34 24 per cent	45 33 per cent	60 43 per cent

Rabbits were injected intravenously with 8 mgm. per kgm. mapharsen, repeated at hourly intervals for a total of four doses. One hour after the last injection, intramuscular treatment with BAL was begun as indicated in the Table.
* Alive and well after 30 days. Control rabbits, receiving no BAL, died regularly within 1 to 72 hours after the mapharsen injections.

TABLE VII

The efficacy of intramuscular BAL in the treatment of systemic Lewisite poisoning in rabbits

Total BAL administered	Method of administration of BAL					
	Single injection	4 equal injections at 4-hour intervals	4 injections at 4-hour intervals (first injection large and next 3 small)*	10 equal injections at 4-hour intervals	10 injections at 4-hour intervals (first 2 injections large and next 8 small)**	8 equal injections at 2-hour intervals
	Proportion of survivors to total treated					
mgm. per kgm.						
80			3/6	2/8	8/10	2/6
40	4/8	2/8	5/8	2/10	2/6	3/4
20	5/8	6/8	2/8	3/9		2/3
10	0/6	3/8	1/8	2/8		2/4
5	1/4	0/3	1/5			
PD$_{50}$ (approximate dose which saved half of animals)	*mgm. per kgm.* 20	*mgm. per kgm.* 15	*mgm. per kgm.* 25±	*mgm. per kgm.* >80	*mgm. per kgm.* 50±	
Individual doses at PD$_{50}$ level, mgm. per kgm.	20	4, 4, 4, 4	14, 3.5, 3.5, 3.5		2×14 8×3.5	
Estimated margin of safety between the maximal tolerated dose† and the PD$_{50}$ level	3	7	4	<3	3	

Treatment with BAL in peanut oil was begun 2 hours after the subcutaneous injection of 4.5 mgm. per kgm. Lewisite (2×LD$_{50}$, and approximately 1.3×LD$_{100}$ level of the particular lot used). Figures in the body of Table (e.g. 3/8) represent proportion of survivors to total treated.

* First dose 4 times larger than following 3: total amount of BAL injected in this series 10 per cent less than indicated in first column.

** One-fourth of total at each of first 2 injections, and 1/16th at each of following 8: first 2 injections 4 times larger than following 8.

† cf. Tables IV and V.

rabbits poisoned with multiple injections of mapharsen (Tables I and VI), the efficacy of treatment in these animals varied markedly with the total dosage of BAL. Of the various schedules tried, 4 equal injections at 4-hour intervals proved at least as effective, and provided a somewhat wider margin of safety (Table VII) than more condensed or more prolonged schedules of treatment, or schedules in which 1 or 2 large doses were followed by smaller doses. With that schedule of treatment, half the animals could be saved by a total of approximately 15 mgm. per kgm. of BAL, or 4 mgm. per kgm. per injection. This was ⅐ of the maximal tolerated dose of BAL on that schedule of injections, and suggested the feasibility of using BAL systemically in the treatment of severe Lewisite poisoning in man. The satisfactory results listed in Table VII were, however, limited to animals treated 2 hours after the Lewisite injection. When treatment with BAL was begun as late as 6 hours after the injections of Lewisite, no protection was afforded either by a single large injection of BAL, or by 4 injections repeated at 4-hour intervals.

Similar results were obtained in the treatment of animals poisoned with slightly more than the LD$_{95}$ dose of phenyl arsenoxide (hydrolyzed form of the arsenical blister gas phenyldichloroarsine), injected subcutaneously (Table VIII). Treatment with relatively small doses of BAL in peanut oil (1.25 to 5 mgm. per kgm.), begun 2 hours later, and repeated 4 times at 4-hour intervals, followed by single daily injections for 6 days, saved approximately half the animals.

Comparable results with a solution of BAL in peanut oil and benzyl benzoate were obtained in dogs (33). After the application of Lewisite or phenyldichloroarsine to the skin in LD$_{100}$ doses, local treatment with BAL ointment alone, begun 30 or 60 minutes later, was relatively ineffective;

TARLE VIII

The efficacy of intramuscular BAL in the treatment of systemic phenyl arsenoxide (hydrolyzed phenyldichloroarsine) poisoning in rabbits

| Phenyl arsenoxide | BAL dosage | | Number of rabbits | Died | Survived | Percentage of animals surviving* |
	Per injection	Total in first 24 hours				
mgm. per kgm.	*mgm. per kgm.*	*mgm. per kgm.*				
2.2 (1.1 times the LD$_{95}$ dose)	5	20	6	3	3	50
	2.5	10	6	2	4	67
	1.25	5	6	4	2	33
	Controls (No BAL)		7	7	0	0

Treatment with BAL (in peanut oil) was begun 2 hours after the injection of the arsenical, and given in 4 equal doses at 4-hour intervals, followed by single daily injections at the same dosage level, for 3 days.

* Alive and well after 30 days. Untreated controls died in 14 hours to 6 days.

but such local treatment, combined with the intramuscular injection of BAL in peanut oil and benzyl benzoate, saved the majority of the animals. The effective dose (total of 10 to 30 mgm. per kgm.) thus agreed with that found in rabbits poisoned with Lewisite or phenyl arsenoxide. In dogs poisoned by the inhalation of Lewisite at LD$_{80}$ levels, the mortality was not reduced by a single intramuscular injection of BAL in a dose of 20 mgm. per kgm., but the survival time was prolonged. Mortality in dogs similarly poisoned with phenyldichloroarsine was significantly reduced by 2 such doses, administered ½ and 2½ hours after gassing.

MECHANISM OF SYSTEMIC ACTION OF BAL: EFFECT ON ARSENIC EXCRETION

As discussed in the introduction, the antidotal action of BAL has been ascribed (15, 20, 21) to the fact that it reacts with arsenicals to form a stable ring compound, and can thus effectively compete for the arsenical with the thiol groups of tissue proteins. This competition involves two distinct processes: (*a*) combination with the toxic arsenical before it combines with the tissues, and (*b*) removal of arsenic from the tissues after it has already combined, with the formation of BAL-thioarsenite from the tissue protein-thioarsenite, and the release of the tissue thiol groups:

tissue protein-thioarsenite   BAL

BAL-thioarsenite   tissue protein
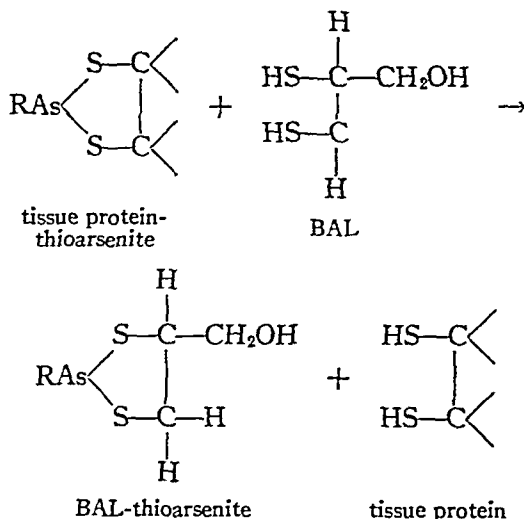
In the systemic use of BAL for the treatment of arsenic poisoning, the latter is perhaps the more important reaction. Highly toxic arsenicals combine with the tissues rapidly (12); and in the case of arsenic compounds used therapeutically, a toxic complication which indicates the use of BAL is presumably the result of a similar combination of arsenic with cellular thiol groups.

That sulfhydryl compounds in general can dissociate arsenic from its combination with cells was indicated by the observation (6) that protozoa (*Colpidium*) immobilized by dichloroarsine could be resuscitated by the addition of monothioethylene glycol, and the similar observation (7, 7a) that *S. pallida* immobilized by a series of phenyl arsenoxides could be resuscitated by cysteine. The even more striking effect of BAL in reviving trypanosomes immobilized by aromatic arsenicals (24) is illustrated in Table IX. Suspensions of trypanosomes (*T. equiperdum*) were exposed to a final concentration of phenyl arsenoxide (1.3 × 10^{-5} molar = 1:460,000) which caused their complete immobilization within less than 1 minute. Within 5 minutes obvious degenerative changes had become evident in most of the organisms, which tended to increase in size, to assume a globular shape, and to become vacuolated; and in the following 30 minutes a significant proportion had completely lysed. At varying intervals after the admixture of arsenical and organisms, aliquot portions were withdrawn and added to varying concentrations of BAL and cysteine. As little as 3 molar equivalents of BAL added to the poisoned

TABLE IX

The resuscitation by BAL and by cysteine of trypanosomes immobilized by phenyl arsenoxide

Time between addition of arsenical and of —SH compound to trypanosomes	Amount of —SH compound added, relative to arsenical (molar)											
	BAL						Cysteine					
	12	6	3	1½	½	1	2500	1250	625	312	156	78
	Percentage of motile organisms 60 minutes after addition of —SH compound											
(—SH compound added to arsenical 10 min. before trypanosomes)			>95	>95	0	0		>95	>95	75	0	0
(—SH compound added immediately after arsenical)			>95	>95	0	0	>95	>95	84	0	0	0
5 min.	>95	>95	92	0			79	77	41	0		
15 min.	73	50	40±	0			20±	20±	6	0		
30 min.	12	15	4	0			0	0	0	0		

Reagents: 4×10^{-5} M phenyl arsenoxide (1 volume); suspension of *T. equiperdum* (5×10^7 per ml.) in 20 per cent rabbit serum and 0.04 M phosphate buffer at pH 7.4 (2 volumes); solution of —SH compound (1 volume). The arsenical used completely immobilized all the organisms within 1 minute, and was 20 times the amount which immobilized half the organisms in 60 minutes.

suspension 5 minutes after the arsenical caused the prompt resuscitation of the trypanosomes. Within a few minutes, more than 90 per cent of the organisms were actively motile, and 92 per cent were still fully motile 60 minutes later. The longer the poisoned suspension was allowed to stand before the addition of BAL, the smaller the proportion which could be so revived. Thus, only 12 to 15 per cent could be resuscitated when BAL was added 30 minutes after the arsenical; and the proportion was actually smaller than indicated, since a significant number had by that time undergone complete autolysis. Although cysteine had qualitatively the same effect, from 200 to 400 times as much was required as in the case of BAL, and the maximum proportion of organisms which could be resuscitated was regularly lower.

The effect of BAL in resuscitating organisms immobilized and poisoned by arsenicals is related to the fact that it abstracts the arsenic from the cells. As is indicated in Table X, actively trypanocidal compounds may be concentrated by the organisms more than 200-fold. On the addition of BAL, the arsenical is rapidly removed, and the concentration in the cells is reduced to a non-lethal level. Since the widely varying trypano-

cidal activity of a series of trivalent arsenicals is determined by the degree to which these compounds are bound by the organisms (13), one may conclude that the demonstrated ability of BAL to remove arsenic from its combination with cell components is the basis of its efficacy in reversing the toxic action of arsenicals.

In the animal body also, the protective action of BAL is associated with an increased excretion of arsenic. Stocken and Thompson (22) demonstrated a 4-fold increase in rate after the local application of BAL to a skin area burned with Lewisite. In rabbits poisoned with phenyl arsenoxide or Lewisite, and to a lesser extent, with mapharsen, we have found a striking increase in the rate of urinary arsenic excretion on systemic treatment with BAL. It had been previously shown in this laboratory (12) that the rate of excretion of arsenicals was related to their toxicity: the more toxic the compound, the slower it was excreted, presumably because of its firmer and more extensive combination with the host tissues. Thus, in the first 24 to 72 hours after the injection of phenyl arsenoxide (0.56 mgm. per kgm. = 0.0033 millimols per kgm.) into rabbits, the hourly urinary excretion was only 0.12 to 0.35 per cent of

the amount injected (Table XI). The administration of BAL to such rabbits, whether intravenously in aqueous solution (Figure 1) or subcutaneously in peanut oil (Figure 2), caused a striking increase in the rate of urinary excretion. The greatest effect was observed when BAL was given immediately after the arsenical, in which case the hourly excretion reached as high as 21.9 per cent, or approximately a *hundredfold* increase over control rabbits.

It is to be noted that the effect of the BAL was temporary. After both intravenous and intramuscular injection, with both aqueous and oil solutions respectively, its action was largely completed within 2 to 4 hours. By that time all the BAL had presumably been either excreted, or metabolized to an inactive form. Further, with multiple injections of BAL, each injection was followed by a definite spurt in urinary excretion. These observations suggested the advisability of administering BAL in repeated small doses, rather than a single massive dose, a procedure already indicated on the basis of minimum toxicity and maximum efficacy (Tables IV to VII).

A single experiment with Lewisite is summarized in Figure 3. BAL in peanut oil (10 mgm. per kgm.) administered intramuscularly 24 hours after the intravenous injection of 0.0033 moles per kgm. Lewisite caused a definite increase in the rate of urinary arsenic excretion, sustained for a period of 2 to 4 hours.

One of many similar experiments with mapharsen is summarized in Figure 4. As is there illustrated, with this compound the effect of BAL on urinary excretion, although definite, was less pronounced than in the case of either phenyl arsenoxide or Lewisite. This probably reflects the several facts (*a*) that much larger doses of

TABLE X

The removal of arsenic from trypanosomes by BAL and by cysteine

Arsenical		Total number trypano-somes	—SH compound added	Time between addition of As and centrifugation	Percentage of motile organisms at time of centrifugation	As content, γ		Ratio of trypanosome As concn.**
Compound used*	Total As added, γ					Trypano-somes	Super-natant	supernatant As concn.
4-CONH₂ phenyl arsenoxide	16.7	1.9×10⁹	0	*min.* 10	0	12.7	6.5	257
			BAL: 1 ml. of 0.02 M solution added 3′ after arsenical	10	>95	2.5	13	28
Phenyl arsenoxide	16.7	2.3×10⁹	0	10	0	9.5	6.0	189
			0	60	0	9.0	7.0	154
			Cysteine: 1 ml. of 0.1 M solution added 10′ after arsenical	60	43	3.5	12.5	33
			BAL: 1 ml. of 0.01 M solution added 10′ after arsenical	60	81	1.17	—	9
3-NH₂-4-OH phenyl arsenoxide (Mapharsen)	13.5	3×10⁹	0	15	0	7.6	5.9†	118
			BAL: 1 ml. of 0.002 M solution added 5′ after arsenical	15	>95	1.9	12‡	15

(Nine ml. trypanosome suspension +1 ml. arsenical solution +1 ml. —SHR or NaCl solution)
* φ=phenyl.
** 3×10⁹ trypanosomes=0.12 ml. Thus, in the experiment with 4-CONH₂ phenyl arsenoxide, 1.9×10⁹ trypanosomes, measuring 0.076 ml., contained 12.7 γ As, or 167 γ per ml. The supernatant fluid contained 0.65 γ per ml., giving a concentration ratio of 257.
† 9.8 ml. of supernatant contained 5.3 micrograms arsenic.
‡ 9.6 ml. of supernatant contained 10.5 micrograms arsenic.

TABLE XI

The effect of BAL on the urinary arsenic excretion in rabbits after the intravenous injection of phenyl arsenoxide

BAL administration (10 mgm. per kgm.)		Rabbit number	Pre-BAL		Post-BAL			Percentage of increase in urinary As excretion caused by BAL
Vehicle for BAL	Route of injection		Time in hours between injections of arsenical and BAL	Hourly As excretion in urine*	Time in hours after BAL injection / Hourly excretion of As in urine*			
					0 to 2	2 to 4	4 to 24	
Aqueous solution	Intravenous	7634	0		7	3	0.36	3900**
		8347	24	0.35	2		0.6	570
		8391	24	0.13	4.8		0.43	3700
		8846	24	0.125	5.1		0.16	4100
Peanut oil and benzyl benzoate	Subcutaneous	9145	24	0.22	8.4	2.1	0.33	3800
		9168	0		21.4	1.4	0.38	11900**
	Intramuscular	9666	0		21.9	11.8	0.33	12200**
		9678	24	0.29	5	5.5	0.25	1700
Controls	No BAL		Time in hours after injection of arsenical					
		N.B.	0 to 24	0.1				
			24 to 48	0.23				
			48 to 72	0.17				
		6274	0 to 4	0.2				
			4 to 24	0.29				
			24 to 48	0.08				
		6460	0 to 48	0.07				
			48 to 72	0.017				

All animals received 0.56 mgm. per kgm. phenyl arsenoxide = 0.25 mgm. per kgm. As = 0.0033 millimols per kgm. All BAL injections were at 10 mgm. per kgm. = 0.08 millimols per kgm.

* Percentage of total amount injected.   Method used for As analysis was that of Magnuson and Watson (34).
** Average hourly excretion in absence of BAL taken as 0.18 per cent of amount injected (cf. column 5).

mapharsen were used than in the case of the other more toxic arsenoxides, and (b) that mapharsen is normally excreted at a faster rate than is either Lewisite or phenyl arsenoxide (12). In consequence, the increased excretion resulting from the administration of BAL was partially obscured by the significant amounts excreted without reference to BAL.

As in the case of microorganisms, the increased rate of excretion of arsenic caused by BAL, and its therapeutic efficacy, are presumably due to the fact that it abstracts the arsenical from an otherwise firm combination with the tissue cells, with the release of vital tissue thiol groups and the excretion of the BAL-thioarsenite. The nature of the chemical grouping in the cells with which the arsenic combines to exert its toxic action has been discussed in a preceding section.

It should be emphasized that thiol compounds, including BAL, successfully reversed the toxic action of arsenicals on spirochetes or trypanosomes only if applied soon after the organisms are immobilized. Beyond that period secondary reactions apparently occur, so that the mere removal of the arsenical does not then suffice to restore cell function (6, 7a). In the animal also, the efficacy of BAL in preventing or reversing the systemic toxic action of arsenicals depended to a large extent on the time elapsed between the application or injection of the arsenical and the administration of BAL. When BAL was injected 5 minutes after a single massive dose of mapharsen all the animals could be saved by a single dose; but when it was administered 30, 60 and 120 minutes after lethal doses of mapharsen, Lewisite, or phenyldichloroarsine, the protective action of the BAL became progressively less striking, and a smaller proportion of the animals could be saved. Six hours after the injection of a lethal dose of Lewisite in rabbits, even intensive and prolonged
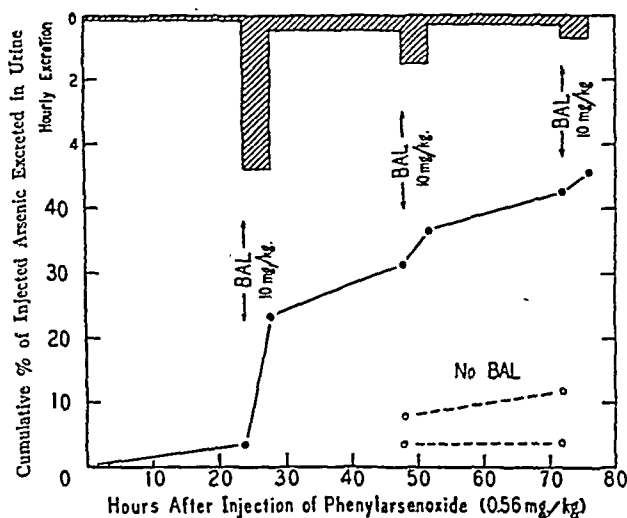
FIG. 1. THE EFFECT OF BAL (INJECTED INTRAVENOUSLY IN SALINE SOLUTION) ON THE URINARY EXCRETION OF PHENYLARSENOXIDE IN RABBITS

A rabbit was injected intravenously with 0.56 mgm. per kgm. phenyl arsenoxide. Twenty-four, 48 and 72 hours later the rabbit was given an intravenous injection of 10 mgm. per kgm. BAL in saline solution. Urine specimens were collected by catheterization just before and 4 hours after each BAL injection. The open circles at the bottom of the figure refer to 2 control rabbits receiving no BAL. The striking effect of BAL on the hourly excretion of arsenic is indicated in the cross-hatched blocks at the top of the figure.

treatment with BAL, at doses approaching the toxic range of the BAL itself, was ineffective (24).

The implication is clear that for optimum results, BAL should be administered as soon as possible after exposure to toxic arsenicals, or after the development of toxic manifestations as a complication of arsenical chemotherapy. In patients heavily exposed to arsenical blister gases, local decontamination with BAL to remove the surface material should be supplemented by its prompt systemic administration to counteract the effects of the material already absorbed.

The effect of BAL on the urinary excretion of arsenic in normal human volunteers, in subjects exposed to an arsenical smoke, and in human cases of arsenic poisoning are discussed in following papers of this series (35, 23). The results obtained with BAL in the treatment of 227 cases of arsenic poisoning (encephalitis, dermatitis, blood dyscrasias, jaundice, fever) will be described elsewhere (32, 35).

SUMMARY

1. 2,3-Dimercaptopropanol ("BAL") injected subcutaneously, intramuscularly, or intravenously in aqueous or propylene glycol solution, proved effective in the treatment of acute and subacute mapharsen poisoning in rabbits.

2. The antidotal action of BAL was referable

Single intravenous injection (0.56 mg/kg.= 0.0033 mmol /kg.)
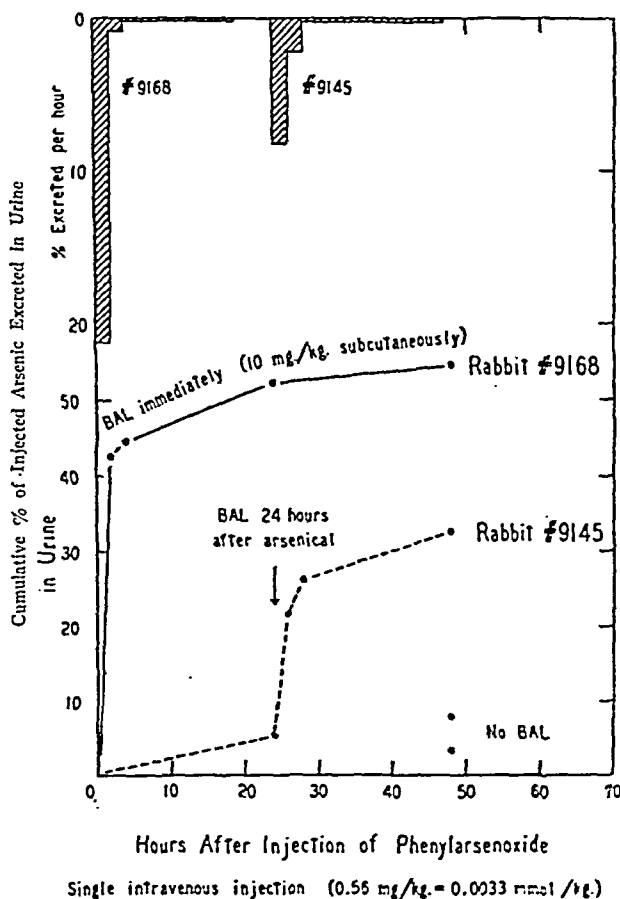
FIG. 2. EFFECT OF BAL (INJECTED INTRAMUSCULARLY IN PEANUT OIL-BENZYL BENZOATE SOLUTION) ON THE URINARY EXCRETION OF PHENYLARSENOXIDE IN RABBITS

Four rabbits were injected intravenously with 0.56 mgm. per kgm. phenyl arsenoxide. One was immediately injected subcutaneously with 10 mgm. per kgm. BAL (5 per cent solution in peanut oil with 10 per cent benzyl benzoate), while the second rabbit received a similar single injection of BAL 24 hours after the arsenical. Urine specimens were obtained by catheterization just before the injection of BAL and 2, 4 and 24 hours later. The open circles at the bottom of the figure indicate the urinary arsenic excretion in the 2 control rabbits receiving no BAL.

to its ability to remove the arsenical from its combination with cells, with the excretion of the stable and relatively non-toxic thioarsenite so formed. Trypanosomes rapidly immobilized and apparently killed by arsenicals were resuscitated on the addition of BAL, due to the removal of the bound arsenic from the cell. Similarly, in rabbits injected with mapharsen, Lewisite or phenyl arsenoxide, the administration of BAL caused a striking increase in the rate of urinary arsenic excretion, in some cases exceeding a hundred-fold.

3. Although BAL was unstable in aqueous or propylene glycol solution, solutions in peanut oil could be sterilized by heat with only slight loss in activity. With the addition of 2 grams of benzyl benzoate for each gram of BAL, the latter was miscible with peanut oil in all proportions.

4. The toxicity of such solutions in peanut oil and benzyl benzoate was determined in relation to the frequency and number of injections, the route of administration, and the concentration of the solution.

5. BAL dissolved in peanut oil and benzyl benzoate injected intramuscularly proved effective in the treatment of mapharsen, Lewisite and phenyl arsenoxide poisoning in rabbits.

6. The widest margin of safety between the effective and toxic levels of BAL so administered was provided by a schedule involving 4 injections at 2- to 4-hour intervals, followed in some cases by daily injections for 6 days. On this schedule, individual BAL doses of 1 to 10 mgm. per kgm. saved approximately half the animals injected with lethal doses of mapharsen, Lewisite or phenyl arsenoxide. Since the maximum tolerated dose of

Single intravenous injection (0.69 mg./kg = 0.0033 mmol./kg.)
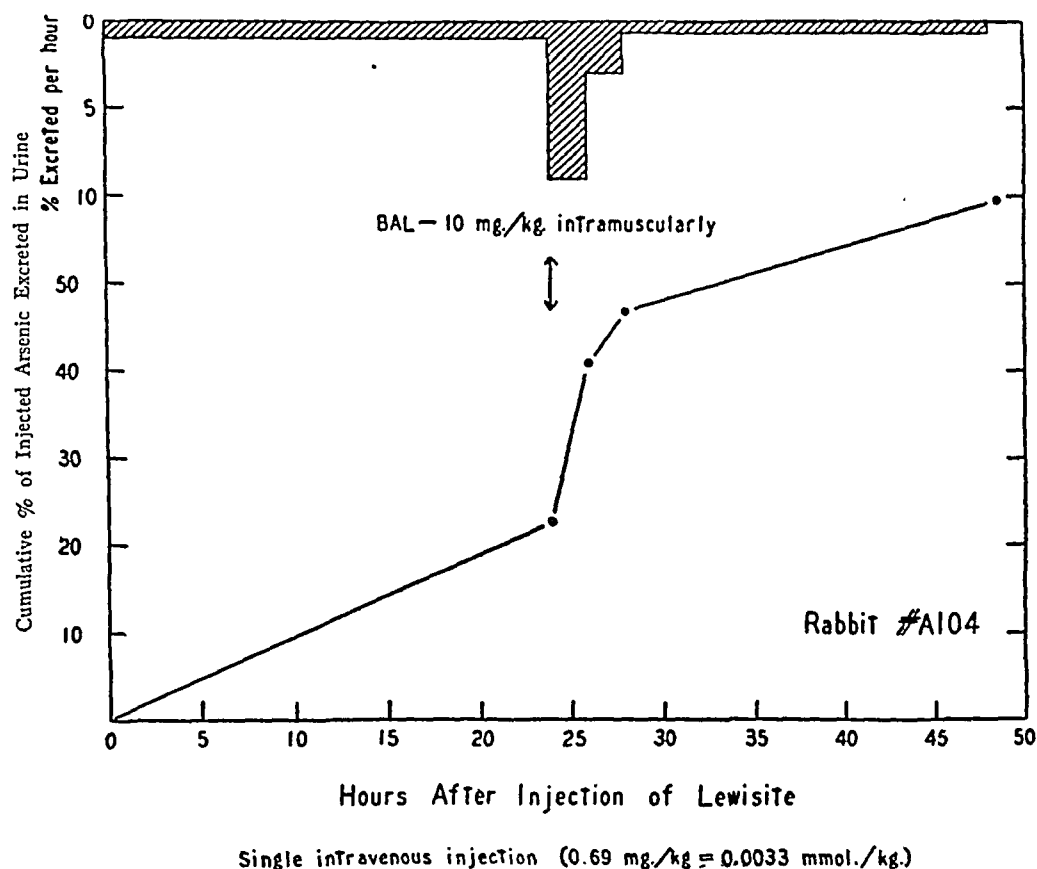
FIG. 3. THE EFFECT OF BAL (INJECTED INTRAMUSCULARLY IN PEANUT OIL-BENZYL BENZOATE SOLUTION) ON THE URINARY EXCRETION OF LEWISITE IN RABBITS

Lewisite was injected intravenously in propylene glycol solution (0.69 mgm. per kgm.). Twenty-four hours later the rabbit was given a single intramuscular injection of 10 mgm. per kgm. BAL (5 per cent solution in peanut oil with 10 per cent benzyl benzoate). Urine specimens were obtained by catheterization just before the injection of BAL, and 4, 8 and 24 hours later.
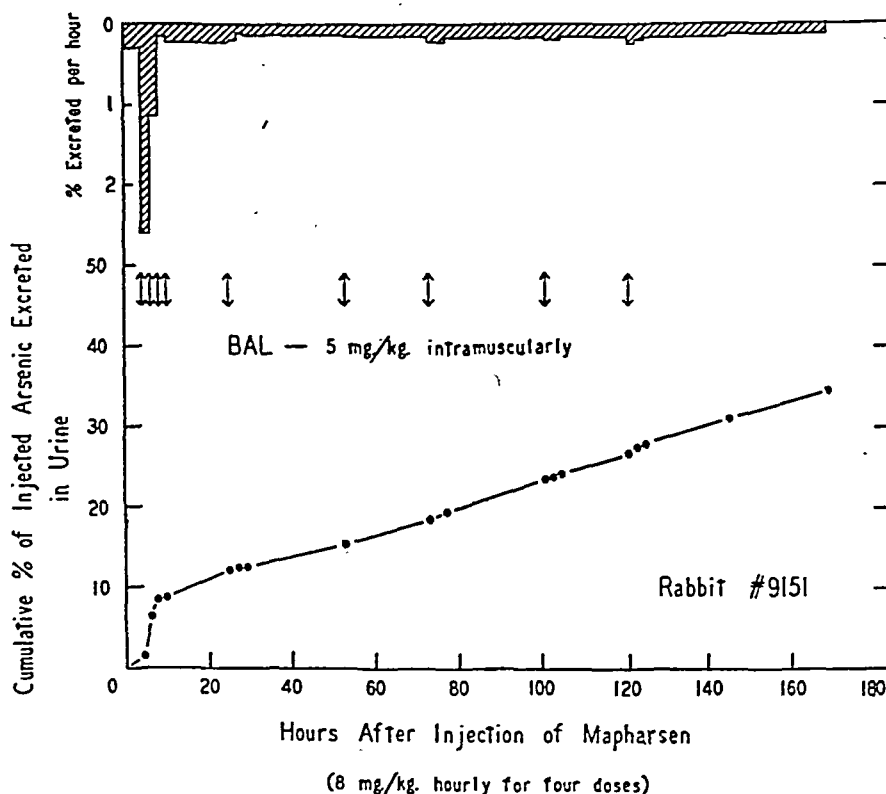
FIG. 4. THE EFFECT OF BAL (INJECTED INTRAMUSCULARLY IN PEANUT OIL-BENZYL BENZOATE SOLUTION) ON THE URINARY EXCRETION OF MAPHARSEN IN RABBITS

Rabbits were injected intravenously with 8 mgm. per kgm. mapharsen, repeated 4 times at hourly intervals. One hour after the last injection of mapharsen the animals received 5 mgm. per kgm. BAL intramuscularly (1 per cent solution in peanut oil with 2 per cent benzyl benzoate). This was repeated 4 times on the first day, and daily thereafter. Urine specimens were obtained by catheterization. The effect of BAL on the hourly urinary excretion of arsenic is indicated in the cross-hatched portion of the figure.

BAL so injected was 35 mgm. per kgm., the margin of safety provided was sufficiently large to indicate the feasibility of its human use.

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CLINICAL USES OF 2,3–DIMERCAPTOPROPANOL (BAL). II. THE EFFECT OF BAL ON THE EXCRETION OF ARSENIC IN NORMAL SUBJECTS AND AFTER MINIMAL EX– POSURE TO ARSENICAL SMOKE

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It has been shown by several investigators (1, 2) that "BAL" (2,3-dimercaptopropanol) accelerates the excretion of arsenic in experimental animals poisoned with various arsenicals.

In man also, the cutaneous application of BAL ointment (3) or the intramuscular injection of a solution in peanut oil and benzyl benzoate (4, 5) in cases of arsenical dermatitis sometimes results in an increased urinary arsenic excretion, as determined by analyses on 12- to 24-hour specimens collected before and after its administration. In view of the short-lived effect of BAL in experimental animals, it is possible that these effects of BAL on urinary arsenic excretion in man would have been more pronounced and more regular had it been possible to obtain urine specimens at 2- to 4-hour intervals.

The present paper represents an attempt to determine the effect of BAL on the normal urinary arsenic excretion in man, as compared with its effect in human subjects exposed for brief intervals to minimal concentrations of an arsenical smoke (diphenylcyanoarsine). By treating the latter group at varying intervals after exposure and by obtaining urine specimens at 2-hour intervals, it was hoped to delimit somewhat more precisely its effect on the urinary excretion of arsenic, and to ascertain whether BAL could be used to confirm suspected minimal exposure to arsenical poison gases. The results might be of significance also in relation to the detection and prevention of industrial arsenic poisoning.

METHODS

1. Normal controls.

Six normal young soldiers served as controls. On the morning of the test, after breakfast, bladders were emp-

tied at 8 a.m. No cigarettes or solid food were allowed during the following 12-hour experimental period. Two hundred ml. of H_2O were thereafter taken by mouth each hour, and 500 ml. of milk at 12 m., and again at 5 p.m.

Urine specimens were collected every 2 hours into Erlenmeyer flasks cleaned with arsenic-free acid. At the end of the third 2-hour period, each man was injected intramuscularly with 3.5 mgm. of BAL per kgm. body weight, administered as a 10 per cent solution in peanut oil with 20 per cent benzyl benzoate as a solubilizing agent (2). Following treatment, the urine was again collected every 2 hours for 6 hours. There were thus a total of 6 2-hour specimens on each volunteer, 3 before and 3 after the injection of BAL.

The arsenic content of the individual specimens was determined by the method of Magnuson and Watson (6). The milk was analyzed and found to be arsenic-free (less than 0.01 micrograms per ml.).

2. Exposure of men to diphenylcyanoarsine (D.C.) smoke.

Twelve men were exposed in a gas chamber for 6 minutes to diphenylcyanoarsine smoke, at a total arsenic concentration of 3.9 mgm. per cu.m. Of this, 1.6 mgm. was present as diphenylcyanoarsine, 0.5 mgm. as other forms of organic arsenic, and 1.8 mgm. as inorganic arsenic.

The exposed men were then divided into 4 groups of 3 men each. Studies similar to those carried out on the 6 normal controls were carried out on one group of 3 men beginning ½ hour after exposure, on a second group 24 hours after exposure, on a third 48 hours after exposure, and on the last group of 3 men 72 hours after exposure. The 4 groups therefore received their BAL 6½, 30, 54 and 78 hours after exposure.

Ten of the 18 injections of BAL were followed by a rise in blood pressure, which returned to normal within 2 hours. Eleven of the BAL injections gave rise to mild to moderate symptoms consisting of nausea, sense of burning in mouth and face, a dry, peppery taste in mouth and salivation. These symptoms disappeared within 2 hours. Significant but temporary local pain followed all injections. The incidence of these symptoms was higher than those reported by other investigators (7, 4) in men injected with a dose of 4 mgm. per kgm. In no instance,

[1,2] Capt., M.C.

TABLE I

Urinary arsenic excretion in 6 normal men

Subject	Before injection of BAL				After injection of BAL			
	2-Hourly excretion of arsenic				2-Hourly excretion of arsenic			
	−6 to −4	−4 to −2	−2 to 0	Average	0 to 2	2 to 4	4 to 6	Average
	mmg.	*mmg.*	*mmg.*	*mmg.*	*mmg.*	*mmg.*	*mmg.*	*mmg.*
Bu	5.2	6.6	6.9	6.2	7.0	9.3	7.3	7.9
Ca	4.0	3.9	7.6	5.2	5.0	5.2	3.7	4.6
Ch	2.6	3.5	2.3	2.8	4.2	4.8	2.9	4.0
De	1.9	3.9	2.6	2.8	2.9	4.4	3.5	3.6
Gr	4.4	5.3	4.3	4.7	6.0	4.1	2.9	4.3
Wi	2.0	3.3	3.2	2.9	4.6	5.3	5.1	5.0
Average	3.3	4.4	4.5	4.1	4.9	5.5	4.2	5.0

however, were the symptoms sufficiently severe to have contraindicated a repeat injection.

EXPERIMENTAL RESULTS

1. Normal controls.

The analytical results in the 6 normal controls are given in detail in Table I. They are graphically shown in Figure 1, in which the excretion in each 2-hour period after the injection of BAL has been plotted relative to the average 2-hourly excretion for that individual prior to the administration of BAL.

It is evident that in 5 of the 6 subjects, the injection of BAL caused a definite if slight increase in the normal arsenic excretion. In 4 of the 5 the excretion reached its maximum in the second 2-hour period after the administration of BAL, and the increase was no longer apparent in the third 2-hour period.

The average absolute increase in arsenic excretion was 0.9 micrograms (standard error 0.1) in the first 2-hour period, 1.4 micrograms (standard error 0.6) in the second, and 0.2 micrograms (standard error 0.7) in the third. The average percentage increase in excretion was 20 in the first 2-hour period, 37 in the second, and 2 per cent in the third. The changes in the first 2 periods after the administration of BAL, although small, are nevertheless significant.

2. Men exposed to diphenylcyanoarsine.

The 12 men exposed to diphenylcyanoarsine were divided into 4 groups of 3 each, which were treated with BAL at different times after exposure. The analytical data with respect to arsenic excretion are given in Tables II and III.

The average 2-hour urinary excretion of arsenic ½, 24, 48 and 72 hours after exposure to diphenylcyanoarsine was 13.3, 13.6, 7.8 and 6.0 micrograms respectively (Table II), as compared with a level of 4.1 in a control group

of normal subjects not exposed to the arsenical smoke. The administration of BAL in each of these groups was regularly followed by an increased urinary excretion of arsenic, reaching its maximum in 9 of the 12 subjects in the second rather than the first 2-hour period. In all but 3 of the 12 subjects the effect of BAL had essentially worn off in 4 hours, and in 6 of the 12, the third 2-hour period actually showed a decreased rather than increased excretion as compared with the average 2-hour excretion before BAL.

The absolute increase in micrograms excreted per 2-hour period was maximal in those treated with BAL 6½ hours after exposure, where it averaged 5.2, 9.0 and 3.3 in 3 successive 2-hour specimens (Table III). In those treated 30 hours after exposure, the corresponding values were + 4.9, + 4.7, and − 2.8 (*i.e.*, an actual decrease in the last 2-hour period) ; 54 hours after exposure the effect of BAL was even less, the increase averaging 1.8, 1.9 and 1.8 micrograms respectively; and 78 hours after exposure the change caused by BAL was + 2.2, + 2.7, and − 0.4 micrograms in 3 successive 2-hour specimens. When the results were expressed on a percentage basis (Figure 3), and referred to the average excretion immediately preceding the administration of BAL as 100, it was found that the injection of BAL at 6½ hours after exposure was followed by a percentage increase of 39, 68, and 25 per cent in 3 successive 2-hour specimens. When treatment was delayed for 30 hours after exposure, the corresponding values were + 20, + 19 and − 20 per cent. In subjects treated 54 hours after exposure, there was an increase of 23, 24, and 23 per cent in 3 successive 2-hour specimens; and the corresponding values in these treated 78 hours after exposure were + 37, + 45 and − 7 per cent. These figures are to be compared with the percentage changes of + 20, + 37, and + 2 per cent in 6 normal subjects similarly treated.

The analytical data are graphically summarized in Figures 2 and 3. Each curve in those figures refers to a group of 3 subjects treated with BAL, at varying times after exposure, and each point on the curves is the aver-

FIG. 1. THE EFFECT OF A SINGLE INTRAMUSCULAR INJECTION OF BAL (3.5 MGM. PER KGM.) ON THE URINARY EXCRETION OF ARSENIC IN 6 NORMAL SUBJECTS

The excretion in 3 successive 2-hourly periods immediately preceding the administration of BAL was averaged, and the data of Table I referred to that average as 100 per cent.

TABLE II

Urinary arsenic excretion in 12 men exposed to diphenylcyanoarsine smoke

Group	Time after exposure at start of experimental period	Subject	Before Injection of BAL				After Injection of BAL			
			2-Hourly excretion of arsenic				2-Hourly excretion of arsenic			
			−6 to −4	−4 to −2	−2 to 0	Average	0 to 2	2 to 4	4 to 6	Average
	hours		*mmg.*	*mmg.*	*mmg.*	*mmg.*	*mmg.*	*mmg.*	*mmg.*	*mmg.*
I	½	Gu	13.0	11.2	14.6	12.9	10.4	17.2	14.0	13.9
		Ho	14.8	15.0	16.7	15.6	30.4	31.2	22.0	27.9
		On	13.2	11.0	9.7	11.3	14.6	18.4	11.8	14.9
		Average	13.7	12.4	13.9	13.3	18.5	22.3	15.9	18.9
II	24	Ca	15.9	14.8	14.2	14.9	17.2	17.9	11.7	15.5
		Sh	7.3	10.4	9.3	9.0	11.5	13.2	8.1	10.9
		Te	15.2	15.0	20.3	16.8	20.5	17.6	12.5	16.9
		Average	12.8	13.4	14.6	13.6	16.4	16.2	10.8	14.5
III	48	Bu	10.8	9.9	9.7	10.1	12.2	10.2	8.8	10.4
		De	5.8	5.3	4.8	5.3	7.9	9.0	6.2	7.7
		Gr	8.2	8.4	6.9	7.8	8.8	9.8	13.9	10.8
		Average	8.3	7.9	7.1	7.8	9.6	9.7	9.6	9.6
IV	72	Ca	6.2	6.0	8.3	6.8	9.1	10.5	7.0	8.8
		Fo	5.9	6.0	4.3	5.4	8.3	8.7	5.5	7.5
		Kr	5.1	6.8	5.8	5.9	7.3	6.8	4.4	6.2
		Average	5.7	6.3	6.1	6.0	8.2	8.7	5.6	7.5

TABLE III

The increase in the urinary excretion of arsenic after a single intramuscular injection of BAL
(3.5 mgm. per kgm.) in 12 men exposed to diphenylcyanoarsine smoke

(After data in Table II)

Time between exposure to diphenylcyanoarsine and injection of BAL	2-hourly urinary arsenic excretion before BAL*	Change in 2-hourly urinary arsenic excretion after injection of BAL					
		1st post-BAL period		2nd post-BAL period		3rd post-BAL period	
hours	*mmg.*	*mmg.*	*per cent*	*mmg.*	*per cent*	*mmg.*	*per cent*
6½	13.3**	+5.2	+39	+9.0	+68	+3.8	+25
30	13.6	+2.8	+20	+2.6	+19	−2.8	−20
54	7.8	+1.8	+23	+1.9	+24	+1.8	+23
78	6.0	+2.2	+37	+2.7	+45	−0.4	−7

* Average of 3 successive 2-hour periods.
** This and all other values in table are average of 3 different subjects in each period.

age urinary arsenic excretion of those 3 subjects in a particular 2-hour period. In Figure 2 the results have been plotted in absolute terms, as micrograms of arsenic excreted. In Figure 3, the excretions have been plotted on a percentage basis, taking for the reference value of 100 the average of the three 2-hour periods preceding the administration of BAL.

DISCUSSION

It has been shown that a single intramuscular injection of BAL at a dose of 3.5 mgm. per kgm. increased the urinary arsenic excretion in 5 of 6 control subjects, and 11 of 12 men exposed for 6 minutes to a low concentration of diphenylcyanoarsine smoke. In no instance was the administration of BAL followed by a decreased excretion. In all groups, the maximum increment in urinary arsenic excretion occurred within the first 4 hours, and usually during the second 2-hour period after the injection of BAL. Thereafter, the rate of excretion decreased, so that the average excretion during the third 2-hour period after BAL was not significantly different from that in the 6-hour control period preceding its administration, and in 8 of the 18 subjects was actually less.
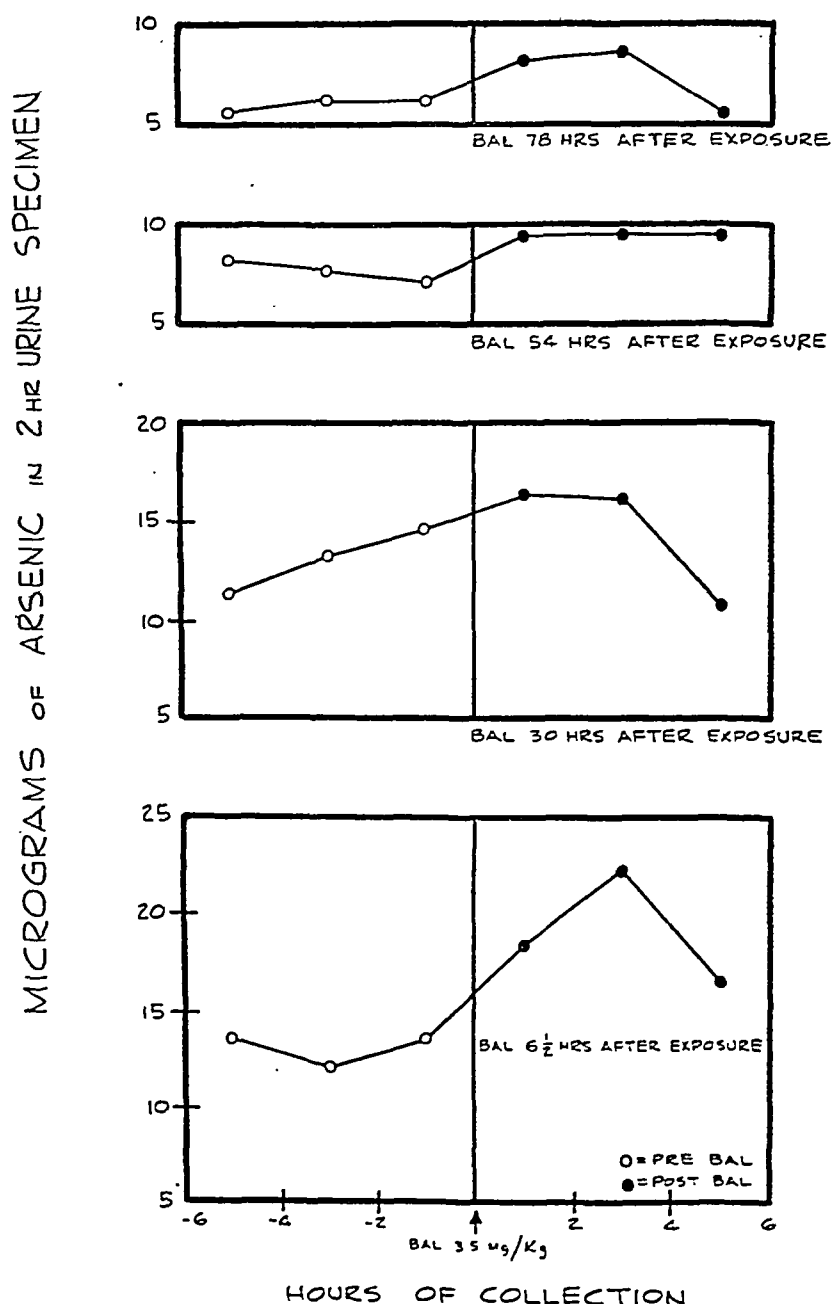
FIG. 2. THE EFFECT OF A SINGLE INTRAMUSCULAR INJECTION OF BAL (3.5 MGM. PER KGM.) ON THE URINARY EXCRETION OF ARSENIC IN 12 SUBJECTS EXPOSED TO DIPHENYLCYANOARSINE SMOKE

Subjects were treated 6½, 30, 54, and 78 hours after exposure. Three urine specimens were collected at 2-hourly intervals for 6 hours before and after the administration of BAL. The plotted values are the average of 3 subjects in each group.

These results are qualitatively similar to those observed in rabbits (2b). The increment in arsenic excretion here observed (up to 100 per cent, averaging approximately 40) did not even approach the 20- to 130-fold increase observed in rabbits poisoned with phenylarsenoxide or Lewisite (2,

BAL 78 HRS AFTER EXPOSURE

BAL 54 HRS. AFTER EXPOSURE

BAL 30 HRS AFTER EXPOSURE

BAL 6½ HRS AFTER EXPOSURE

O = PRE BAL
● = POST BAL

PERCENT OF AVERAGE 2 HOURLY URINARY ARSENIC EXCRETION

BAL 3·5 Mg/Kg
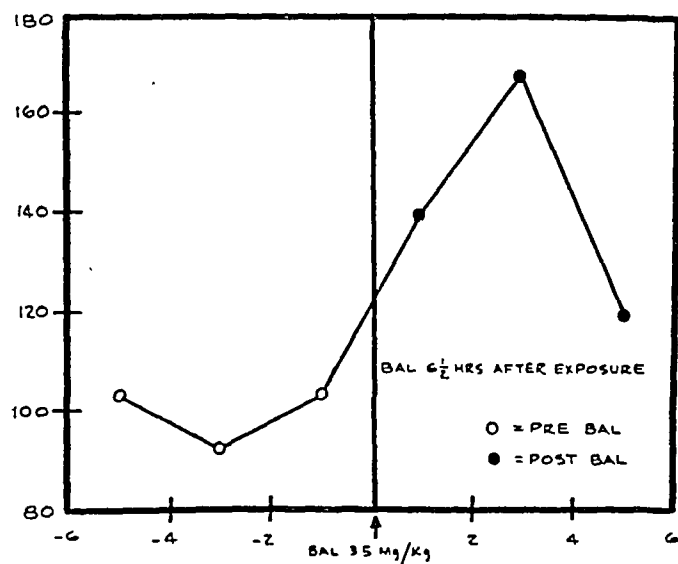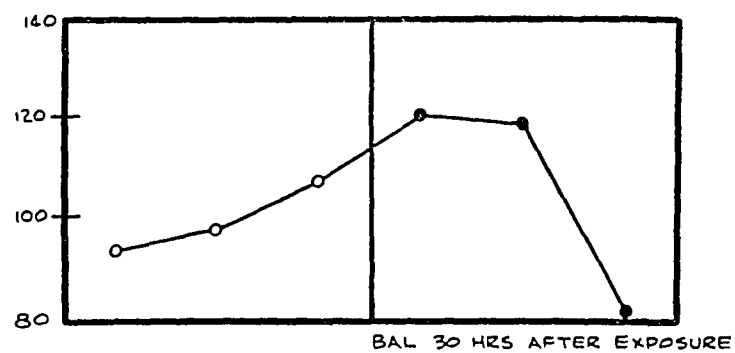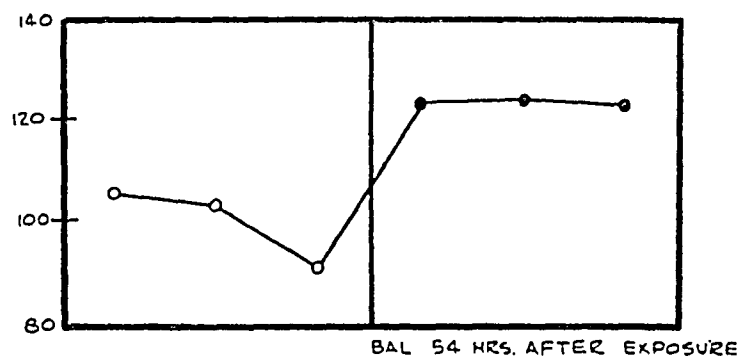
HOURS OF COLLECTION

4). The difference may be referable to the larger doses of arsenical administered in the experimental animals (0.56 to 0.69 mgm. per kgm.), the varying method of its administration (intramuscular or subcutaneous instead of by inhalation) and the larger doses of BAL employed (10 mgm. per kgm. instead of 3.5 mgm. per kgm.). Despite these differences, in man as in animals the effect of BAL on arsenic excretion had disappeared within 4 hours.

It follows from these observations that when solutions of BAL in peanut oil and benzyl benzoate are used therapeutically, the optimum interval to be allowed between injections, *i.e.*, the longest interval which permits a continuous effect on arsenic excretion, is approximately 4 hours as originally recommended (2, 4). It follows, also, that the previously reported data with respect to the effect of BAL on the urinary excretion of arsenic in cases of arsenical dermatis, based on pooled 12- to 24-hour specimens, do not necessarily reflect the maximum effect of BAL on the rate of that excretion. A similar series of patients should be studied on the basis of 2-hour specimens collected for a period of *e.g.* 6 hours before and 6 hours after a therapeutic injection of BAL.

It would appear from the data herein reported that the determination of the urinary arsenic is a simple and reliable criterion for the detection of abnormal exposure to arsenic in man. The effect of BAL in promoting excretion may be used as a supplementary procedure, of particular value if it is used soon after exposure.

SUMMARY

1. In 12 men exposed for 6 minutes to minute concentrations of an arsenical smoke, there was an immediate increase in the urinary excretion of arsenic, which gradually fell thereafter from an initial average of 6.8 micrograms per hour to an average of 3 micrograms per hour 72 hours after exposure. The corresponding value in a group of 6 control subjects was 2 micrograms per hour.

2. In both groups, those exposed to arsenic as well as the normal controls, a single intramuscular injection of BAL at a dose of 3.5 mgm. per kgm. administered as a 10 per cent solution in peanut oil and benzyl benzoate, was followed by a significant and regular increase in the rate of urinary arsenic excretion. The effect usually reached a maximum within 2 to 4 hours, and was usually not demonstrable thereafter. The largest increase, both absolute and relative, was observed in the group of subjects treated with BAL soon (6½ hours) after exposure to the arsenical smoke.

3. The significance of these observations with respect to the optimum time interval between successive therapeutic injections of BAL, and its use to detect abnormal exposure to arsenic, are discussed in the text.

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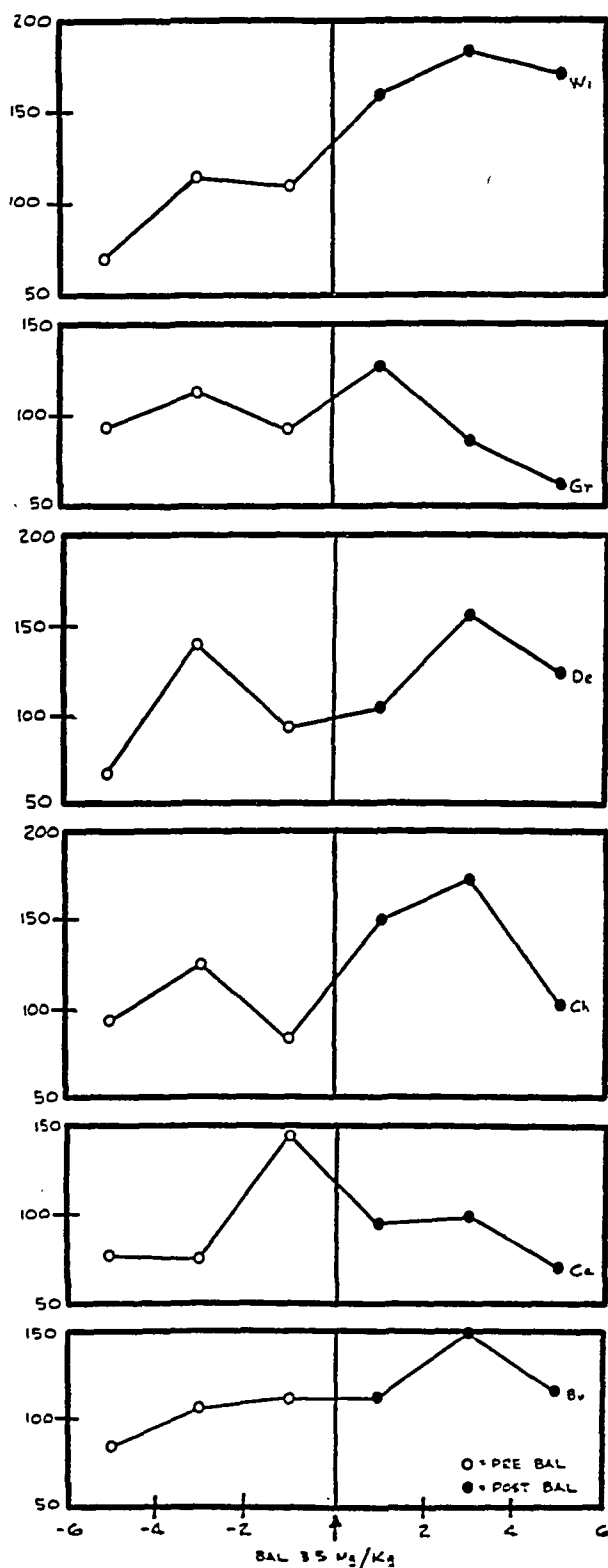
FIG. 3. THE EFFECT OF A SINGLE INTRAMUSCULAR INJECTION OF BAL (3.5 MGM. PER KGM.) ON THE URINARY EXCRETION OF ARSENIC IN 12 SUBJECTS EXPOSED TO DIPHENYLCYANOARSINE SMOKE

Subjects were treated 6½, 30, 54, and 78 hours after exposure. The excretion in 3 successive 2-hourly periods immediately preceding the administration of BAL were averaged for each group of 3 subjects, and the data of Table II referred to that average as 100 per cent.

CLINICAL USES OF 2,3-DIMERCAPTOPROPANOL (BAL). III. STUDIES ON THE TOXICITY OF BAL ON PERCUTANEOUS AND PARENTERAL ADMINISTRATION [1]

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Ointments and vehicles containing BAL may, on occasion, have to be used over extensive skin areas, and also repeatedly, within relatively short periods of time. For this reason it was essential to ascertain the possible systemic toxic effects of BAL on repeated application to extensive skin areas in man. Moreover, before the parenteral administration of BAL could be adopted as a method for treating certain metallic poisonings, it was imperative to ascertain the toxicity of BAL preparations when administered parenterally in human subjects.

The following are among the important findings which form the background of the present studies. Early experiments in laboratory animals had demonstrated that the external application of BAL to the skin can produce systemic toxic manifestations and even lethal effects (1 to 4). With 1 early sample of BAL, doses of 600 mgm. per kgm. killed about ½ the mice when applied externally. It has been shown, however, that early lots of BAL were considerably more toxic than the standard product of later manufacture.

Also, there was evidence that significant quantities of BAL penetrate the grossly normal human skin quite rapidly when BAL is applied, either undiluted or in various vehicles. This is shown by the almost immediate whealing and erythema produced, and by certain systemic effects which have been observed following external application. The latter include the benefits produced on arsenical dermatitis in skin areas distant from the actual sites of application (5), and the occasional malaise, nausea, etc. seen during the course of experimental application of BAL to the skin.

Thus Stocken and Thompson (1) applied 0.5 ml., and later 1 ml., of undiluted British BAL to the arm of a normal test subject. With both doses there followed an evanescent "scarlatiniform rash" on the area of exposure; and with the larger dose there were slight conjunctivitis, lachrymation and salivation.

I. STUDIES ON THE TOXICITY OF BAL ON PERCUTANEOUS ADMINISTRATION [2]

Method

In a first experiment, 5 generally healthy, young, white, male subjects received 3 grams of BAL 10 per cent in K-Y jelly (in 3 subjects), and BAL 10 per cent in petrolatum (in 2 subjects) rubbed into the skin of the arms and forearms. Transitory erythema and whealing were produced in several of the subjects, but no apparent systemic ill effects were observed.

Five generally healthy, young, white, male subjects were selected for a second experiment. General physical examinations, complete blood counts, urine analyses, blood pressure and temperature measurements were carried out on these subjects for 24 hours preceding the application of BAL. All findings were within normal ranges. Each subject received 1 ounce of BAL 5 per cent in K-Y jelly rubbed in vigorously over the neck, entire back, shoulders, chest, abdomen and upper arms. The amount of BAL applied was 1.5 grams. None of the applied material was deliberately removed.

Results

Varying degrees of local skin reactions were noted at the sites of inunction, ranging from severe generalized whealing over the entire area of application in one subject to no visible skin reaction whatsoever. Two other subjects showed moderate degrees of evanescent urticaria at the sites of application. In the subject with severe whealing, the lesions subsided gradually during the 20 hours following inunction, and at 36 hours there was only slight erythema to be seen.

One of the subjects, about 20 minutes after the inunction, complained of feeling faint and dizzy and had to lie down. While he did not lose consciousness entirely, he appeared like a person in

a faint. The pulse was scarcely perceptible, but when it could be counted was regular, and not accelerated. There was extreme pallor and the skin was moist. There were no convulsive phenomena, but as precaution 1½ grains (about 0.1 gram) of phenobarbital were given 40 minutes after the inunction. Recovery was gradual, but the subject appeared well and was smoking a cigarette within 1½ hours after inunction.

This subject gave a history of previous fainting spells and it was not possible to ascertain to what degree, if any, the shock-like condition was attributable to psychogenic factors. The close atmosphere and the strong, and to many people disagreeable, smell of BAL in the room in which the experiment was performed, may also have been factors in producing the reaction in this subject.

General physical examinations, blood counts, urine analyses and blood pressure and temperature measurements were carried out in all subjects at regular intervals during the 48 hours after the inunctions. There were no abnormal findings and no changes which could be attributed to the BAL inunctions in any of the subjects.

II. STUDIES ON THE TOXICITY OF BAL ON INTRAMUSCULAR ADMINISTRATION [3]

Method

1. Subjects: These experiments were carried out on 34 healthy male volunteers ranging from 17 to 29 years in age, and from 55.3 to 86.0 kgm. in weight. The average weight was close to 70 kgm.

2. Material: A solution containing American Reference Standard BAL 10 per cent, benzyl benzoate 20 per cent in peanut oil (prepared and filled in ampules by Hynson, Westcott and Dunning Inc.).

3. Mode of Administration: By deep intramuscular injection, alternately in the upper outer quadrant of the right and left gluteal areas. A new site was chosen for each injection.

4. Dosage: Three series of tests were performed, each one utilizing a different dosage and/or time schedule. These 3 series were as follows:

Series I
5 subjects, 4 injections (one every 4 hours) as follows:

(1) 1.5 ml. (150 mgm. BAL) at 8 a.m.
(2) 2.0 ml. (200 mgm. BAL) at 12 noon
(3) 2.8 ml. (280 mgm. BAL) at 4 p.m.
(4) 3.0 ml. (300 mgm. BAL) at 8 p.m.

Series II
24 subjects, 8 injections (on 2 successive days) as follows:

1st day (1) 1.5 ml. (150 mgm. BAL) at 8 a.m.
(2) 2.0 ml. (200 mgm. BAL) at 12 noon
(3) 2.5 ml. (250 mgm. BAL) at 4 p.m.
(4) 3.0 ml. (300 mgm. BAL) at 8 p.m.

2nd day (5) 3.5 ml. (350 mgm. BAL) at 8 a.m.
(6) 3.5 ml. (350 mgm. BAL) at 12 noon
(7) 3.5 ml. (350 mgm. BAL) at 4 p.m.
(8) 3.5 ml. (350 mgm. BAL) at 8 p.m.

In Series I and II the highest single dose was 5.5 mgm. per kgm. of body weight. The highest total dose in 12 hours was 22 mgm. per kgm. On the second day in Series II the average single dose was 5 mgm. per kgm., and average total dose in 12 hours was 20 mgm. per kgm.

Series III
5 subjects, 4 injections as follows:

(1) 3.5 ml. (350 mgm. BAL) at 9:30 a.m.
(2) 3.5 ml. (350 mgm. BAL) at 11:30 a.m.
(3) 3.5 ml. (350 mgm. BAL) at 3:30 p.m.
(4) 3.5 ml. (350 mgm. BAL) at 7:30 p.m.

In Series III the highest single dose was 6.3 mgm. per kgm. of body weight. The highest total dose in 10 hours was 25.3 mgm. per kgm., and in 2 hours 12.7 mgm. per kgm. The average total dose in 10 hours was 20.8 mgm. per kgm., and in 2 hours 10.4 mgm. per kgm.

5. Examination: In Series I, II and III the following examinations were carried out:

(a) General physical examination.
(b) Measurements of pulse rate, respiration rate and blood pressure before and after injections or as required.
(c) Observations of objective and subjective changes in general.

In Series I and II the following additional examinations were carried out:

(d) Measurement of temperature.
(e) Complete urinalysis before and after each day's course of injections.
(f) Complete blood counts before and after course of injections.
(g) CO_2 combining power [4] and levels of reducing substances of blood (Folin-Wu method) before and after each day's course of injections.

In 12 subjects of Series II the following additional examinations were carried out:

(h) Icteric index of blood and urobilin tests of urine after the course of injections.

Results

Local effects

Beginning a few minutes after the injection, there were varying degrees of pain in the gluteal area around injection sites and some radiating pain and stiffness of the leg. The pain and stiffness were also variable in degree, sometimes moderately severe and persisting through the following 24 hours. There was slight tenderness of most injection sites at 24 hours. No severe local reactions, such as redness, swelling, infiltration or abscess formation, were noted.

General signs and symptoms

The series of injections were completed as planned in all volunteers except subject H. V. A. In this subject the injections were discontinued after the second injection on the second day because of the somewhat threatening character of the reactions. A few subjects complained of slight headache, slight nausea and slight burning of the mouth at even the lowest dosage. Unequivocal complaints generally began at the dose of 250 mgm. of BAL per injection, i.e. at a dose of about 3.6 mgm. per kgm. The significant manifestations set in within a few minutes after the respective injection, usually reached a maximum between 10 and 30 minutes after injection, and then generally subsided rather rapidly. The effects listed in approximate order of frequency were as follows:

(1) Nausea (and, in 11 instances, vomiting)
(2) Headache
(3) Burning sensation of lips, mouth, throat, constricted feeling and sometimes pain of throat, chest and/or hands
(4) Conjunctivitis, tearing, rhinorrhea and salivation
(5) Tingling of the hands
(6) Burning sensation of penis
(7) Sweating of forehead, hands, etc.
(8) Abdominal pain.

In Series I and II the symptoms, when they appeared after a certain dose, for example, 250 mgm. of BAL, usually reappeared at the subsequent injections, but there was no general increase in the severity of the manifestations on successive injections, and little or no evidence of cumulation. The one possible exception is the

case of H. V. A. described in detail below. In some cases there was perhaps a suggestion of increasing tolerance, later injections of the same dose sometimes producing fewer complaints than preceding ones.

However, in Series III distinct evidence of cumulative toxicity could be observed. In all 5 subjects the second injection, i.e. the one given within 2 hours of the first dose, produced signs and symptoms of much greater intensity than any of the other administrations. This cumulative effect was no longer evident at the third injection 4 hours later, nor at the fourth injection given after another 4-hour interval. Thus the first, third and fourth injections of Series III produced reactions of the same degree as those observed in Series I and II after doses of 350 mgm., while the second injection of 350 mgm. in Series III apparently produced additive effects with the BAL remaining from the first injection, and resulting in the following more severe toxic sequelae:

(a) Burning sensation of lips, mouth, throat (5 out of 5 subjects)
(b) Conjunctivitis, tearing, rhinorrhea, salivation (5 out of 5 subjects)
(c) Abdominal pain, moderate to very severe (4 out of 5 subjects)
(d) Headache (3 out of 5 subjects)
(e) Constricted feeling, burning pain of throat and chest (2 out of 5 subjects)
(f) Tremors and shakiness (2 out of 5 subjects)
(g) Nausea (1 out of 5 subjects)
(h) Lower back pain (1 out of 5 subjects)
(i) Uncontrollable laughing (1 out of 5 subjects).

Laboratory findings

Urine: One subject who had a trace of albumin before the first injection developed 2 plus albumin and a few casts at the end of the second day of injections (subject H. V. A.—see below).

Eleven subjects developed reducing substances in the urine during the series of injections (Benedict's Test). In 6 of these, there were only traces of reducing substances, while in 5, reactions were 1 to 2 plus.

All other urinary findings were normal throughout.

Blood: Blood counts showed no significant abnormalities.

The levels of reducing substances in the blood were all within normal limits.

There was no evidence that the injection produced a reduction in the CO_2 combining power.

The icteric indices measured were all within normal range.

Clinical findings

Temperatures (oral) : These were all within the normal range.

Pulse rates: There was slight transitory acceleration of the pulse rate in 2 subjects following the injections; and in one instance the pulse dropped to 48 a few minutes after the injection (this individual also presented cold sweating of hands and forehead).

Respiration rates: In general, the respirations tended to increase slightly in frequency within 15 to 20 minutes after the injections (maximum was 26 per minute in one subject).

Blood pressures: All subjects had normal blood pressure at the beginning of the tests. There was a distinct tendency for both the systolic and disastolic pressures to rise slightly within 30 to 40 minutes after the injections. How uniform this reaction was, is not certain since the blood pressure readings in the first 17 subjects were not taken at the 30- to 40-minute period. In Series I and II, the blood pressures tended to return to normal within 60 to 90 minutes after the injections. The one exception was H. V. A., whose pressure was elevated for over 2 hours. In Series III the blood pressures were all within the normal range within 3 hours after the BAL injection. Among those subjects in whom the blood pressure was taken at 30 to 40 minutes, 14 of 17 subjects showed significant elevations.

Blood Pressures

Series II	Before start of experiment	Maximum observed
	120/70	144/94
	120/76	164/106
	110/70	164/110
	104/80	136/90
	98/60	144/90
	130/80	150/86
	130/80	150/112
	120/80	144/104
	126/84	220/140
	110/60	146/100
	124/174	200/120
	130/74	144/92
	126/80	150/104
	118/70	146/96

Tests for systemic sensitization to BAL

Fifteen of the original 29 subjects were available for repetition of intramuscular injections of BAL solution at 15 to 27 days after the last previous intramuscular injection of BAL 10 per cent solution. These subjects each received readministrations of BAL as follows: 1.0 ml. of BAL 10 per cent (100 mgm. BAL) in benzyl benzoate-peanut oil solution, deep intramuscularly, followed 2 to 2½ hours later by an injection of 3.5 ml. (350 mgm. BAL).

The examinations carried out at the time of readministration included general physical examination, measurements of pulse rate, respiration rate and blood pressure before and after injections or as required, and observation of objective and subjective changes in general.

There were no signs or symptoms of systemic hypersensitivity or sensitization. The injections were tolerated in a manner analogous to that exhibited by these same subjects at the time they had received their first injections of the equivalent amounts 15 to 27 days previously.

It is perhaps noteworthy that among the 15 subjects reinjected, there were 4 who had acquired skin sensitization as evidenced by the positive patch tests to BAL (6). As stated, these subjects showed no evidence of systemic hypersensitivity. Moreover in no instance did the intramuscular reinjection of 460 mgm. of BAL produce a cutaneous reaction or dermatitis in these patch-test positive subjects.

COMMENT

The studies on percutaneous absorption of BAL indicate that under the conditions of the studies presented, no significant systemic toxic manifestations occurred from the topical application of BAL preparations. The single exception was the subject who fainted after application of 1 ounce of BAL 10 per cent in K–Y jelly, and who had a previous history of occasional fainting spells. It appears very possible that the faint was purely psychogenic. The strong fumes of BAL in the room may have had something to do with the reaction. There were no convulsive phenomena, which are considered characteristic of BAL intoxication.

The varying degrees of transitory local urticarial and erythematous reactions which were noted in many of the inuncted subjects cannot be considered as pathologic or abnormal responses. This form of reaction corresponds to that seen after inunction of other primary urticariogenic agents, such as pilocarpine, atropine, histamine, codeine, etc.

The studies on toxicity of BAL after intramuscular injection indicate that some transitory

toxic manifestations do occur at doses of approximately 3 to 4 mgm. of BAL per kgm. Even 4 doses, each of about 5 mgm. of BAL per kgm., administered at 4-hour intervals, produced no severe or lasting damage in the average normal individual; with the 5 mgm. per kgm. dosage given 4 times at 4-hour intervals, there was no evidence of cumulative toxicity. However, when the second dose of 5 mgm. per kgm. was given after a 2-hour, instead of a 4-hour interval, definite signs of cumulative, although not conspicuously dangerous, toxicity were noted.

Based on these and coordinated studies, the dosage of 4 mgm. of BAL per kgm. given in 4 doses at 4-hour intervals, was adopted for the treatment of systemic arsenic poisoning (7). This dosage schedule appears well within the safe range established in our studies.

In considering our results and before drawing practical conclusions from them, it should be pointed out that all our studies described in the present report were conducted on subjects who were not suffering from arsenical or other metallic poisoning. When BAL is used under practical circumstances, it will almost always be in individuals who are suffering from systemic poisoning due to arsenic or other metals. Therefore, the possibility must be considered that tolerance to BAL may be affected by the presence or absence of arsenical or other metallic poisoning.

SUMMARY AND CONCLUSIONS

1. Approximately 0.3 gram of BAL applied to the skin in the form of BAL 10 per cent in K–Y jelly, or BAL 10 per cent in petrolatum, produced no signs or symptoms in 5 healthy, white, male subjects except for transitory local erythema, whealing, and itching at the site of application.

2. Approximately 1.7 grams of BAL applied to large areas of the skin in the form of a 5 per cent concentration in K–Y jelly produced no convulsive phenomena, blood changes, kidney or other damage, and no other forms of systemic poisoning in any of 5 healthy young male subjects.

However, one subject became faint within 20 minutes after the application. The possibility that this fainting spell may have been caused by psychogenic rather than toxic factors is suggested by a previous history of fainting spells in this subject.

There were various degrees of local whealing, ranging from scarcely visible isolated evanescent wheals in some subjects, to severe generalized whealing of all the areas receiving the inunction in one subject. In this last subject the wheals persisted for about 20 hours. The local skin effects were not incapacitating in any of the 5 subjects.

3. A solution containing 10 per cent BAL and 20 per cent benzyl benzoate in peanut oil was injected according to various dosage schedules in a total of 34 human volunteers. There was considerable variation in the individual response, but in general, toxic manifestations appeared at a dose of approximately 3.6 to 5.5 mgm. per kgm. These manifestations included nausea, vomiting, headache, burning sensation of lips, mouth and throat, feeling of constriction and sometimes pain in throat and chest, burning and tingling of extremities, conjunctivitis, tearing, rhinorrhea, salivation, sweating of forhead and hands, abdominal pain, and general agitation. Local pain at the site of injection and muscle spasm of the leg were also quite common. Perhaps the most significant finding was elevation of systolic and diastolic blood pressure within about ½ hour after the injection.

4. Except for 1 subject in whom some of the ill effects persisted for over 24 hours, the subjects had returned to normal within 45 minutes to 2 hours after the injections. No regular cumulative effects were noted when 4 doses of approximately 5 mgm. per kgm. were given in 4 successive injections 4 hours apart. However, when the first 2 doses were given with an interval of only 2 hours, distinct cumulative toxicity was observed.

5. Reinjections of a total average dose of 6.2 mgm. BAL per kgm. administered 15 to 27 days after the last previous injection elicited no signs of systemic sensitization.

6. Attention is called to the fact that these studies were carried out in volunteers who were not suffering from arsenical or other metallic poisoning. The presence or absence of such poisoning may or may not be a factor in determining the tolerance of BAL.

7. It is concluded that topical application of BAL preparations, at least within the dosages employed in our studies, is a safe procedure. It is furthermore concluded that while toxic manifestations

occur after the intramuscular administration of BAL, 4 doses each of about 5 mgm. of BAL per kgm., administered at 4-hour intervals, will produce no severe or lasting damage in the average normal individual. When the first 2 doses are given only 2, instead of 4 hours, apart, cumulation occurs, and more severe symptoms can be expected, although these too are transitory, and are followed by relatively prompt recovery in normal individuals.

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CLINICAL USES OF 2,3–DIMERCAPTOPROPANOL (BAL). IV. PHARMACOLOGIC OBSERVATIONS ON BAL BY INTRA-MUSCULAR INJECTION IN MAN [1]

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The present report deals with a study [2] of the effects of 2,3-dimercaptopropanol (BAL) in humans. It was designed to explore the range of doses which may be safely administered to humans in relation to the schedules of doses which have been found effective in the treatment of Lewisite poisoning in animals.

SUBJECTS

The experiments were carried out in 9 subjects, 8 females and 1 male, 7 colored and 2 white. Their ages ranged from 18 to 49. They all had secondary or tertiary lues. One had diabetes and another hypertension, and a third, rheumatic heart disease. Three were under active antiluetic treatment; the remaining ones had not received treatment for at least several months.

PLAN

In each of 4 cases the experiments were carried out in the outpatient clinic, the patient leaving the clinic after symptoms had subsided. In each of 5 cases the patient was placed in the hospital, and was discharged on the day following the last dose. The experiment was so conducted as to eliminate anticipation or fear as a factor in the subjective symptoms.

American Reference Standard BAL was used in a 10 per cent solution in peanut oil containing 20 per cent benzyl benzoate (Hinson, Westcott and Dunning). It was injected deeply into the gluteal muscles.

In the general plan the subject was weighed and placed in bed or on an examining couch, where he remained throughout the experiment. The blood pressure, the heart rate, and in some cases the respirations were recorded at approximately 10-minute intervals for 30 minutes prior to the injection, and at approximately 15-minute intervals for about an hour after the injection. Symptoms were recorded as they occurred until the effects had completely or nearly completely disappeared.

RESULTS

Dosage. The schedules of dosage and the results are summarized in Tables I and II. All doses were administered in terms of mgm. per kgm. of body weight. Single doses varied from 3.0 to 8.0 mgm. per kgm.; and total amounts at each injection, from 133.5 to 640 mgm. (1.3 to 6.4 ml. of the 10 per cent solution). In those cases in which more than 1 injection was given in a day, the total doses varied from 8.0 to 20.0 mgm. per kgm. The latter amounts were administered in a period of from 8 to 10 hours.

In the dosage range employed in these experiments (3.0 to 5.0 mgm.) distinct cumulation occurred when doses were given at intervals of 30 minutes. The 30-minute interval, however, greatly reduced the toxicity, since 8.0 mgm. per kgm. at one time produced severe symptoms, while the same amount given in a dose of 5.0 mgm. and 3.0 mgm. with an interval of 30 minutes, produced relatively mild symptoms in the same person.

Cumulation also occurred when the second dose was given at an interval of 2 hours. It may be fairly marked, as shown by case Li Co, in which slight symptoms from the first dose of 5.0 mgm. became very severe symptoms when the same dose was repeated in 2 hours.

A 3-hour interval between doses seemed to permit negligible cumulation, although we had only 1 case (An Ma) on this schedule. With the 4-hour interval between doses there was no cumulation.

Effects. A single dose of 3.0 mgm. per kgm. produced no symptoms. A single dose of 5.0

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mgm. per kgm. produced practically no symptoms in half of the cases (4 out of 8) ; and in the remaining ones, symptoms of moderate severity. After a single dose of 8.0 mgm. per kgm. the symptoms were among the most severe.

Following the injection of BAL intramuscularly, symptoms appeared in from 5 to 30 minutes. They gradually developed in intensity, reaching a plateau which lasted about 30 minutes, and then subsided gradually. In some, the entire course of symptoms lasted only about 15 minutes, while in others the symptoms persisted for as long as 2 hours.

The heart rate was usually accelerated moderately during the peak of the symptoms. A rise of the blood pressure was one of the most regular effects. It was seen in 7 out of 9 cases. It was usually present after the second dose of 5.0 mgm., although occasionally after the first dose. Both the systolic and diastolic pressures rose. The increase ranged from 14 to 52 mm. Hg systolic, and from 10 to 40 mm. Hg diastolic. The course of the blood pressure change usually paralleled the symptoms. The only subject in whom the blood pressure failed to rise was the one with hypertension (An Mo). In this case the blood pressure fell. The patient started in the control period as a hypertensive in the range of 180/90, and

TABLE I

Effects of 10 per cent solution of BAL in various dosage schedules by intramuscular injection in man

Case	Date	Dose of BAL		Interval since last dose	Effect on			Symptoms		
		per kgm.	Total		Heart rate	B. P. (mm. Hg.)		Intensity 0 to 4+	Onset	Duration*
						S	D			
Cl Wa	4/18	mgm. 3.0	mgm. 177.3		0	0	0	0	min.	min.
	4/21	5.0	295.0		0	0	0	0		
		5.0	295.0	30 min.	+8	+32	+18	++	10	43
El De	4/20	5.0	400.0		0	0	0	±		
		3.0	240.0	30 min.	0	+32	+38	+	18	131
	4/21	8.0	640.0		+30	+28	+14	++++	8	52
Et Wo	4/19	5.0	245.0		+8	0	0	+	25	30
		3.0	141.0	32 min.	+20	+30	+32	++++	5	59
Ma Jo	4/18	3.0	133.5		0	0	0	0		
Id Vi	4/29	5.0	320.0		+14	+26	+20	++	30	70
		5.0	320.0	2 hrs.	+24	+32	+36	++++	17	40
		5.0	320.0	4 hrs.	+24	+30	+24	+++	15	50
An Mo	5/1	5.0	320.0		0	−12	0	0		
		5.0	320.0	2 hrs.	0	−44	−38	++	12	46
		5.0	320.0	4 hrs.	0	+8	+12	+	30	21
		5.0	320.0	4 hrs.	0	−16	0	0		
Li Co	5/3	5.0	300.0		+12	+14	+20	+	30	80−
		5.0	300.0	2 hrs.	0	+52	+40	++++	15−	29
		5.0	300.0	4 hrs.	+12	+16	+12	+	25	15
		5.0	300.0	4 hrs.	0	0	0	±	14	
An Ma	5/4	5.0	284.0		0	+10	+14	0		
		5.0	284.0	2 hrs.	0	+42	+28	±	33	15
		5.0	284.0	3 hrs.	0	+36	+26	+	15	30
		5.0	284.0	3 hrs.	−14	−12	−8	0		
Is Bl	5/5	5.0	293.0		+24	+14	+38	++	17	27
		5.0	293.0	2 hrs.	+24	+40	+28	++++	7	37
		5.0	293.0	4 hrs.	+16	0	+14	++	16	50
		5.0	293.0	4 hrs.	+28	+10	+10	+	26	28

* In most instances mild and vague symptoms continued after the last observation and were off gradually.
S (systolic); D (diastolic).

TABLE II

Symptoms produced by BAL in man

Case	Date	Dose of BAL	Interval since last dose	Symptoms									
				Nausea	Vom-iting	Salivation	Eye	Pain	Anxiety	Unrest	Paresthe-sias	Local pain	Warmth
Cl Wa	4/18	*mgm. per kgm.* 3.0		0	0	0	0	0	0	0	0	0	0
	4/21	5.0		0	0	0	0	0	0	0	0	0	0
		5.0	30 min.	0	0	0	+	++	+	++	+	0	0
El De	4/20	5.0		±	0	0	0	0	0	0	0	0	0
		3.0	30 min.	+	0	+	0	+	0	+	+	+	+
	4/21	8.0		++++	+	+	0	++++	++++	++++	+	0	0
Et Wo	4/19	5.0		0	0	0	0	0	0	0	+	+	0
		3.0	32 min.	0	0	++++	0	+	++++	++++	+	0	+
Ma Jo	4/18	3.0		0	0	0	0	0	0	0	0	0	0
Id Vi	4/29	5.0		+	++	+	0	++	++	++	+	0	0
		5.0	2 hrs.	++	+	++	0	++++	++	++++	++++	+	0
		5.0	4 hrs.	++	++	+	0	+++	+	+++	0	+	0
An Mo	5/1	5.0		0	0	0	0	0	0	0	0	0	0
		5.0	2 hrs.	0	0	++	0	0	0	0	++	0	0
		5.0	4 hrs.	0	0	+	0	0	0	0	+	0	0
		5.0	4 hrs.	0	0	0	0	0	0	0	0	0	0
Li Co	5/3	5.0		0	0	+	0	+	0	0	0	+	0
		5.0	2 hrs.	++++	0	+	0	++++	0	++++	+	0	0
		5.0	4 hrs.	0	0	0	0	+	0	0	0	0	0
		5.0	4 hrs.	0	0	+	0	0	0	0	0	0	0
An Ma	5/4	5.0		0	0	0	0	0	0	0	0	0	0
		5.0	2 hrs.	0	0	0	0	0	0	0	±	0	0
		5.0	3 hrs.	0	0	0	0	+	0	0	+	0	+
		5.0	3 hrs.	0	0	0	0	0	0	0	0	0	0
Is Bl	5/5	5.0		+	+	++	0	++	0	0	++	++	0
		5.0	2 hrs.	+	+	++	++	0	0	0	++++	++	+
		5.0	4 hrs.	+	+	++	0	0	0	0	++	++	0
		5.0	4 hrs.	+	+	+	0	0	0	0	0	++	0

ended after the fourth 5.0 mgm. dose of BAL in 10 hours in the range of 138/70. There were no symptoms associated with this fall in the blood pressure.

There is some indication of the development of tolerance to BAL since, as may be seen in Table II, the fourth, and sometimes the third, dose of 5.0 mgm. produced less severe symptoms than the first.

Symptoms. The pattern of symptoms varied from case to case (see Table II). Vomiting was usually associated with the most severe reactions. In the course of the more severe reactions, the patients showed paresthesias (burning or tingling of the nose, eyes, mouth or skin), pain (involv-ing the limbs, jaws, abdomen, chest and head), lacrimation, blepharospasm, and salivation. An indescribable sense of illness developed with extreme unrest and apprehension. Occasionally the patient perspired freely and complained of being very warm. As the symptoms subsided, most patients with the more severe reactions complained of weakness and fatigue. At 4 hours after the 5.0 mgm. doses they were free of systemic symptoms.

While most doses caused some pain during the injection, a moderately tender area in the region of the injection, still present some hours later, was encountered in 4 of the 9 cases.

The complete protocols of the experiments are appended.

Schedules. Single doses of BAL of from 5 to 8 mgm. per kgm. intramuscularly in man produced reactions which did not appear to be dangerous, so also for total doses of 20 mgm. per kgm. in fractions of 5 mgm. over a period of 8 hours.

A single dose of 5 mgm. per kgm. caused no symptoms in 50 per cent of the cases, negligible effects in 25 per cent, and slight and fleeting symptoms in the remaining 25 per cent. The course of the reaction is fairly brief, lasting approximately an hour. The indications are that a schedule of dosage involving 5 mgm. per kgm. for 4 doses at intervals of 3 hours is likely to show little cumulation. In such a schedule, the effects of the first dose are not likely to become greater as a result of its repetition. The effect of the third and fourth dose may actually be less. Shorter intervals with such doses, namely 2 hours or less, result in cumulation.

The review by Waters and Stock (1) indicates that others have obtained substantially similar results (2, 3).

SUMMARY AND CONCLUSIONS

1. The toxic effects of BAL by intramuscular injection were studied in 9 human subjects with secondary or tertiary lues.

2. The results are based on 28 injections of single doses of from 3 to 8 mgm. per kgm.

3. In man BAL produces paresthesias (burning or tingling of the nose, eyes, mouth, and skin), perspiration and sense of warmth, pain (limbs, jaws, abdomen, head), lacrimation, blepharospasm, salivation, vomiting, unrest, apprehension, weakness, and fatigue. The heart rate is accelerated and both systolic and diastolic blood pressure usually increased.

4. The minimal dose which produces toxic effects lies between 3 and 5 mgm. per kgm. A single dose of 8 mgm. produces marked symptoms.

5. The effects of doses up to 8 mgm. per kgm. are completely reversible, the reactions lasting only about an hour or two.

6. Doses of 5 mgm. per kgm. may be given at intervals of three hours during the course of one day without significant cumulation.

PROTOCOLS

Patient: Cl Wa; female; colored; age 18; wt. 59.1 kgm.
    Lues; active treatment.
4/18/44

Time	Blood pressure	Heart-rate	Respir-ation	Remarks-Symptoms
10:40	110/74	108	22	
10:48	110/76	104	22	
10:55	106/72	98	22	
10:57	BAL, 3 mgm. per kgm. (1.8 ml.), muscle, local pain lasting 1 min.			
11:05	108/78	104	24	
11:07	106/74	102	20	
11:18	108/76	100	21	
11:28	108/78	96	22	
11:42	104/72	92	24	No complaints offered at any time, "I feel fine except my hip is stiff from needle," fell asleep during observations.
4/21/44				
1:46	116/74	76		
1:57	120/68	74		
2:16	116/80	88		
2:25	BAL, 5 mgm. per kgm. (2.95 ml.), right gluteus, no local pain.			
2:38	110/74	88		
2:48	106/72	86		
2:55	BAL, second dose as above, left side, no local pain.			
2:56	118/78	84		
3:05	126/84	96		"Feel fine except for medicine in my mouth," "pepper in eyes and mouth."
3:10	152/98	96		About the same.
3:20	130/90	94		Seems weak, restless, says chest hurts, cries.
3:38	128/80	80		Better, can hardly keep eyes open because of smarting.
3:48	116/76	76		Better, eyes almost normal.

Patient: El De; female; colored; age 42; wt. 80 kgm.
    Lues; no treatment.
4/20/44

Time	Blood pressure	Heart-rate	Respir-ation	Remarks-Symptoms
9:40	132/80	78	22	
9:50	124/82	78	22	
9:55	130/90	72	20	
9:59	138/88	70	24	
10:01	BAL, 5.0 mgm. per kgm. (4.0 ml.) muscle, buttock, local pain during injection only.			
10:10	130/90	76	20	
10:22	134/94	72	24	
10:30	128/86	68	24	"Little indigestion."
10:31	BAL, 3 mgm. per kgm. (2.4 ml.), muscle, buttock, immediate local pain.			
10:41	134/92	64	18	"Indigestion."
10:49	132/90	76		Salivation, "indigestion" worse, face and jaws "feel funny," burning in gums.
11:02	156/110	80		
11:06				Warmth in mouth and head.
11:10	164/104	80		Nausea, slightly restless, "I feel miserable."
11:20				Pain in left arm.
11:21	166/118	80		
11:30	130/100	72		Symptoms subsiding.
11:40	130/92	68		All symptoms still present but intensity much less.

4/21/44

Time	Blood pressure	Heart-rate	Respir-ation	Remarks-Symptoms
1:55	142/96	66		Moderate soreness of right hip from previous inject. No swelling.
2:02	134/94	66		
2:32	134/90	64		
3:00	BAL, 8 mgm. per kgm. (6.4 ml.), muscle, left buttock.			
3:02	122/80	72		Soreness during injection.
3:08				"Feels it in mouth," spits.
3:10	126/88	72		No symptoms.
3:15	138/90	72		No symptoms.
3:21	148/88	76		Salivation, itching in jaw, pain in left elbow, extremely restless, nausea.
3:36	170/110	96		Strange sensations in limbs, sensation of stiffness in jaws, "feels miserable all over."
3:43				Still feels bad, insists on walking about, aches all over.
3:50	160/94	88		Pain in both arms and in neck, "would rather have a baby," no eye signs.
4:00	140/88	78		Distinctly improved, vomits.
4:05				Much better and insists on going home, gait normal, some nausea.

Patient: Et Wo; female; colored; age 26; wt. 49 kgm.
    Lues; active treatment.
4/19/44

Time	Blood pressure	Heart-rate	Respir-ation	Remarks-Symptoms
9:40	100/64	72	22	Patient lies comfortably.
9:50	98/62	70	20	Patient lies comfortably.
9:58	96/60	70	20	Patient lies comfortably.
10:00	BAL, 5 mgm. per kgm. (2.5 ml.), muscle, left buttock, immediate pain.			
10:06	92/62	72	20	
10:17	92/60	80	18	Still some local pain in leg.
10:25				Nose stings, "head feels funny."
10:30	94/64	80	22	
10:32	BAL, 3 mgm. per kgm. (1.4 ml.), muscle, right buttock, immediate local pain lasting 1 minute.			
10:37	110/70	80	24	Pain in head, mouth burns, "I feel sick."
10:40				Profuse salivation, prefers to sit.
10:45	132/100	100		"Very sick," unrest, squirms, "I feel nervous," profuse salivation.
10:46				Improving.
10:47				"Lots better," "feel warm," "short of breath."
10:52	130/96	88		Very restless, complains of feeling "very sick," "throat very dry".
10:55		98		Shakes and shivers.
10:58				Less disturbed.
11:01		92	26	Feels cold, no lacrimation.
11:05				Improving.
11:10				Pain in shoulders, "most of misery gone."
11:11		76	26	"I feel worse again."
11:15	110/82			Improving, quieter.
11:20	120/76	68		Nervous, feels weak, premature beats, given phenobarbital gr. ¾.
11:25				"Feel stronger now."
11:30	110/68	66		"Feel strong enough to go home now," seems well.
11:36		64		Normal.

Patient: Ma Jo; female; colored; age 25; wt. 44.5 kgm.
Lues; active treatment; rheumatic heart disease.
4/18/44

Time	Blood pressure	Heart-rate	Respiration	Remarks-Symptoms
9:34	124/84	76	19	Patient lies comfortably.
9:40	130/82	76	16	Patient lies comfortably.
9:48	126/84	76	16	Patient lies comfortably.
9:59	BAL, 3 mgm. per kgm. (1.3 ml.), muscle, buttock, no local pain.			
10:04	124/82	82	18	
10:11	130/86	88	17	
10:21	126/86	84	18	
10:28	130/84	78	18	
10:43	122/82	81	16	
11:00	122/82	78	16	No complaints at any time, "I feel excellent."

Patient: Id Vi; female; white; age 34; wt. 64.5 kgm.
Lues; no treatment.
4/29/44

Time	Blood pressure	Heart-rate	Remarks-Symptoms
3:55	106/74	80	
4:00	106/74	76	
4:05	BAL, 5 mgm. per kgm. (3.2 ml.), muscle, buttock.		
4:15	106/76	80	
4:25	106/78	84	
4:35	132/94	88	Nausea, "nose tickles, pressure in ears" (paresthesias), "feel funny."
4:36			Vomits, "chills."
4:40			Vomits.
4:45	120/82	94	Salivation, abdominal cramps, other symptoms begin to subside.
5:00	122/82	84	
5:15	108/76	88	
5:30	106/70	82	Nervous, headache, given phenobarbital 0.1 and aspirin 0.3.
5:45	98/74	76	Symptoms entirely gone.
6:00	98/62	76	No symptoms.
6:03	BAL, second dose as above. Local pain lasting one minute.		
6:20	124/96	84	Cramps, "pressure in ears," paresthesias, tingling in gums, marked salivation, nausea.
6:33	136/98	100	Intense cramps, "pressure in chest," restlessness, anxiety.
6:37			Vise-like squeezing in chest, "I can't stand it any more," vomits.
6:40	128/92	94	Lacrimation, diffuse erythematous rash over shoulders.
6:50	116/90	96	Symptoms subsiding, pentobarbital sodium 0.1.
7:00	118/86	88	Much better but very fatigued.
10:00	107/70	70	Tenderness in left buttock, otherwise no symptoms.
	BAL, third dose as above, pain during injection.		
10:15			Slight nausea, sneezing.
10:35			Copious vomiting.
10:37	128/90	91	
10:50	137/94	94	Marked headache, no other symptoms.

Patient: An Mo; female; colored; age 49; wt. 64.2 kgm.
Lues; no treatment.
5/1/44

Time	Blood pressure	Heart-rate	Remarks-Symptoms
10:43	170/88	92	Patient lies comfortably.
10:53	178/90	98	Patient lies comfortably.
10:58	180/90	96	Patient lies comfortably.
11:00	BAL, 5 mgm. per kgm. (3.2 ml.), muscle, right buttock, no pain.		
11:15	176/90	92	No effect.
11:30	174/88	88	No effect.
11:40	176/88	82	No effect.
12:45	166/88	88	No effect.
1:00	BAL, second dose as above, in left buttock, local pain during injection lasting 1 minute.		
1:12	188/110	104	"Strange feeling."
1:20			Tingling in mouth, salivation.
1:35	192/98	96	"Feel very sick."
1:40	142/72	82	Improving, profuse salivation.
1:55	148/84	76	Much improved.
1:58			Normal.
5:00	170/84	84	Normal.
5:03	BAL, third dose as above in left buttock.		
5:18	166/78	80	No effect.
5:33	164/74	80	"Tickling" in mouth.

5:44	174/90	84	Itching in mouth, salivation.
5:54			Normal.
8:46	158/78	78	Normal.
8:54	BAL, fourth dose as above in right buttock.		
9:20	152/74	76	No effect.
9:35	138/70	76	No effect.
9:45	136/72	72	No effect.

Patient: Li Co; female; colored; age 43; wt. 60 kgm.
    Lues, no treatment; diabetes; receives insulin.
5/3/44

Time	Blood pressure	Heart-rate	Remarks-Symptoms
6:40	144/80	64	
6:48	144/82	60	
6:58	138/80	60	
7:00	BAL, 5 mgm. per kgm. (3.0 ml.) muscle, buttock, ocal pain at site of injection.		
7:15	144/86	64	
7:30	158/102	76	"Ache all over."
7:45	152/90	72	Salivation.
8:50	138/68	60	No symptoms.
9:00	BAL, second dose as above.		
9:15	176/104		Restless, groaning.
9:28	196/120		Restless, writhing, groaning, crying, nausea, salivation, pain, "I can't stand it."
9:44	138/80	70	Tingling in hands, otherwise symptoms gone.
12:45	136/74	68	No symptoms.
1:00	BAL, third dose as above.		
1:15	128/68	68	No symptoms.
1:25	144/80	76	Slight pain in head and arms, "not bad."
1:40	144/80	72	Almost no symptoms, fatigued.
4:50	130/68	80	No symptoms.
5:00	BAL, fourth dose as above.		
5:14	128/68	88	Slight salivation.
5:28	134/76	80	No symptoms.
5:42	134/74	72	No symptoms.

Patient: An Ma; white male; age 41; wt. 56.8 kgm.
    Lues; no treatment.
5/4/44

Time	Blood pressure	Heart-rate	Remarks-Symptoms
10:33	138/78	96	
10:43	136/80	100	
10:55	138/84	92	
10:58	BAL, 5 mgm. per kgm. (2.8 ml.), muscle, buttock.		
11:14	134/84	88	
11:29	146/98	84	
12:40	148/92	92	
12:55	148/96	88	No symptoms at any time.
12:57	BAL, second dose as above.		
1:15	148/98	82	
1:30	180/112	88	Slight paresthesias in hands, face, eyes, nose and mouth, "like somebody is touching me."
1:45	164/104	88	Practically no symptoms.
3:55	138/88	96	No symptoms.
3:58	BAL, third dose as above.		
4:15	174/110	94	Very slight paresthesias like those at 1:30.
4:30	166/100	100	Symptoms more intense, restless, pain, sweating.
4:45	154/94	96	Symptoms subsiding.
6:55	132/80	82	No symptoms.
6:58	BAL, fourth dose as above.		
7:15	128/74	78	
7:30	124/70	84	
7:40	124/76	92	No symptoms after this dose.

Patient; Is Bl; female; colored; age 41; wt. 58.6 kgm.
    Lues; no treatment.
5/5/44

Time	Blood pressure	Heart-rate	Remarks-Symptoms
6:47	122/64	72	
6:52	122/70	68	
6:58	118/72	68	

6:59	BAL, 5 mgm. per kgm. (2.9 ml.), muscle, left buttock.		
7:16	132/80	74	"Feel funny and sick all over," salivating.
7:29	130/110	84	Headache, vomited, perfuse salivation, pain in injected leg.
7:43	122/88	92	Better but still feeling sick.
8:53	132/84	96	No symptoms.
8:59	BAL, second dose as above in right buttock.		
9:06			"Funny feeling," salivation.
9:15	154/98	92	"Hot," symptoms more severe than after first dose.
9:30	162/100	96	Lacrimation, symptoms slightly less.
9:43	154/80	88	Vomited, but otherwise better.
12:53	118/70	88	Hungry, no symptoms except pain in both buttocks.
12:58	BAL, third dose as above in left buttock.		
1:14	116/70	86	"Funny feeling" starts, salivation.
1:30	120/86	88	Symptoms slightly more intense.
1:50	116/72	84	Nausea and vomiting, symptoms subsiding.
2:25			No symptoms, effects slightly less than from previous doses.
4:58	128/70	98	
5:02	BAL, fourth dose as above in right buttock.		
5:14	120/70	100	No effect.
5:28	124/72	96	"Feel something coming on," salivation.
5:38			Vomiting.
5:40	134/82	98	Feels better.
5:56	128/74	88	No symptoms other than pain at the site of injections, last dose caused least symptoms.

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CLINICAL USES OF 2,3–DIMERCAPTOPROPANOL (BAL). V. SKIN SENSITIZATION TO BAL [1]

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The possibility that BAL (2,3-dimercaptopropanol) (1) might be used extensively on human skins naturally raised the question of its potentilities as a skin sensitizer. Kidd in 1942 (2) reported on deliberately produced skin sensitization to BAL in guinea pigs. In 1942 we observed and described what was to our knowledge the first human case of skin sensitization to BAL (3). Somewhat later, Sparks and Levi (4) found evidence of sensitization to BAL in 13 out of 32 volunteers whose skin had been burned with liquid Lewisite, and subsequently treated with 1 or several applications of BAL solution applied to the chemical burns. Davis (5) reported dermatitis due to BAL, or a complex containing BAL, in 11 men (18 per cent) of a series of 61 volunteers who each had one of several Lewisite burns treated with BAL solution. However, it is noteworthy that in 8 of the 11 volunteers in Davis' group, the dermatitis was not confined to the BAL-treated sites, but was present also at the other sites to which Lewisite, but not BAL, had been applied.

The evaluation of the sensitizing capacity of BAL, on the basis of the experimental work cited above, was complicated by the fact that in all the observations on human beings the application of BAL had in each instance been preceded by the local application of some other chemical agent, which had produced an inflammatory reaction in the skin. The experiments which are the subject of this report were designed to answer the following questions: Is BAL alone a sensitizing agent when repeatedly applied to a normal skin area of human subjects; if so, what is the incidence of such sensitization; and is the incidence of sensitization produced by BAL alone significantly different from the incidence of sensitization obtained by the application of BAL to skin areas which have been damaged previously by some other chemical agent, such as Lewisite or liquid mustard gas?

EXPERIMENT I—PRODUCTION OF DELIBERATE SENSITIZATION OF HUMAN SKIN TO BAL

Subjects and procedure

One hundred and two human volunteers received skin applications of BAL as follows:

Group A: 35 human subjects were given a supply of 5 per cent BAL in a grease ointment.

Group B: 35 human subjects were given a supply of 5 per cent BAL in a carbowax ointment.

Group C: 32 human subjects were given a supply of 5 per cent BAL in ethylene glycol.

All subjects were intelligent and cooperative; and all were carefully shown how to rub a small amount of the BAL preparation, which had been handed to them, into a skin area of approximately 5×10 cm. on the flexor aspect of the left forearm. No definite instructions as to the length of the rubbing or the amount to be applied were given, but the amount which was suggested and used in demonstration was about 0.3 to 0.4 gram of BAL ointment, or 2 drops of the BAL solution delivered with a medicine dropper. The subjects themselves repeated the rubbings to the same area daily for a total of 14 applications. Interval readings were made on the 7th day, and the final readings were made on the 15th day. At the start of the experiment all volunteers were handed protocol forms on which they were to note the dates of the applications and the signs and symptoms, if any. The applications were to be stopped as soon as a persistent and definite erythema or other type of eruption appeared. The transitory erythema and whealing (6), which are quite regularly observed 15 minutes to 2 hours after application of BAL, were discounted.

Results

Ninety-one of the 102 subjects who started the experiment were still available for reexamination after 15 days. Three of these 91 subjects could not be included in the evaluation of the data, since the applications either had been stopped prematurely or had been carried out too infrequently and/or irregularly.

Only persistent erythematous and papular local reactions were regarded as evidence that sensitization had taken place.

The degrees of reaction were recorded as ranging from mild to marked as follows:

Mild reactions consisted of a faint but definite erythema and/or a few isolated pinhead-sized papulo-urticarial lesions.

Marked reactions consisted of marked erythema and/or numerous confluent pinhead-sized urticarial and papular lesions.

Moderate reactions were those intermediate between the two first described.

Itching of the affected area was slight to moderate in a few of the volunteers with marked reactions; and was either negligible or absent in all others. In no case did the reactions interfere with the regular activities of the volunteers (studies, drill, physical exercise).

The results in the individual groups were:

Group A (5 per cent BAL in grease ointment): 31 of 35 subjects completed the experiment. Of these 31 subjects, 6 had become sensitized (2 markedly, 1 moderately, 3 slightly).

The dermatitis started in 1 volunteer 7 days after the first application; the dermatitis started in 1 volunteer 9 days after the first application; the dermatitis started in 1 volunteer 11 days after the first application; the dermatitis started in 3 volunteers 14 days after the first application.

Group B. (5 per cent BAL in carbowax ointment): 28 of 35 volunteers completed the experiment. Of these 28 subjects, 4 had become sensitized (2 markedly, 1 moderately, 1 slightly).

The dermatitis started in 1 volunteer 12 days after the first application; the dermatitis started in 2 volunteers 13 days after the first application; the dermatitis started in 1 volunteer 14 days after the first application.

Group C (5 per cent BAL in ethylene glycol): 29 of 32 volunteers completed the experiment. Of these 29 subjects, 6 had become sensitized (2 markedly, 4 slightly).

The dermatitis started in 1 volunteer 10 days after the first application; the dermatitis started in 1 volunteer 11 days after the first application; the dermatitis started in 2 volunteers 12 days after the first application; the dermatitis started in 2 volunteers 14 days after the first application.

Thus, under the conditions of this experiment, 5 per cent BAL in a grease ointment, 5 per cent BAL in a carbowax ointment, and 5 per cent BAL in ethylene glycol produced definite sensitization and sensitization dermatitis in 16 out of 88 human subjects, *i.e.*, in 19 per cent of the subjects exposed. There appeared to be no significant difference in the sensitizing capacity of BAL when used in each of the three vehicles mentioned above.

There was no evidence that the onset of sensitization was earlier in those volunteers in whom marked reactions developed, than in those in whom the eventual reaction was slight.

Clinically the sensitization dermatitis was papulo-urticarial in appearance; definite clinical vesicles were not observed. The eruption did not spread beyond the sites of the deliberate application. In all cases the eruption subsided and disappeared without treatment within a maximum of about 5 days after the last exposure.

EXPERIMENT II—THE SPECIFICITY OF BAL SENSITIZATION; LOCAL DIFFERENCES IN SENSITIVITY TO BAL

Subjects

Nineteen subjects participated in this experiment.

Group A: 6 subjects previously exposed and sensitized. This group consisted of 6 of the subjects who had become most strongly sensitized in Experiment I, and who were selected for further study. The further studies were begun 13 days after the last inunction of BAL-containing material, and at a time when the dermatitis had entirely disappeared in even the strongest reactors. Two of these 6 subjects had become sensitized through application of 5 per cent BAL in grease ointment; 2 through application of 5 per cent BAL in carbowax ointment, and 2 through application of 5 per cent BAL in ethylene glycol.

Group B: 6 subjects previously exposed to BAL but *not* sensitized. This group consisted of 6 subjects who had been included in Experiment I, but who had shown no signs of dermatitis or sensitization after the 14 daily applications of BAL preparations. Two of these had been among those receiving applications of 5 per cent BAL in grease ointment, 2 had received applications of 5 per cent BAL in carbowax ointment, and 2 had received applications of 5 per cent BAL in ethylene glycol.

Group C: 7 subjects not previously exposed to BAL, and presumably not sensitized to that substance. This group consisted of 7 "fresh" subjects who had not been used in any previous experiments and who, to the best of our knowledge, had no previous exposure to BAL or its relatives.

Procedure

Each of the 12 subjects in Groups A and B received an inunction of 5 per cent BAL both to the previously exposed flexor surface of the left forearm (Exp. I), and also to the not previously exposed flexor surface of the right forearm. In each individual the vehicle employed for the BAL was the same as that which had been used in Experiment I in his particular case. The amount of ointment rubbed on the skin of each arm was approximately 0.3 to 0.4 grams, and the amount of liquid was 2 to 3 drops delivered with an ordinary medicine dropper. The duration of each inunction was 30 seconds.

The 7 not previously exposed control subjects (Group C) received inunctions to each forearm in the manner above described; 2 received 5 per cent BAL in grease ointment; 2 received 5 per cent BAL in carbowax ointment; and 2, 5 per cent BAL in ethylene glycol.

In addition to these inunction tests with BAL, other substances chemically related to BAL were studied in order to throw light on the specificity of the BAL reaction. The formulas of these substances are set forth in Table I. They were applied to the backs of each of the 19 volunteers in the form of both scratch and patch tests with the orthodox procedures (7). The scratch tests were read for immediate wheal reactions from 20 to 40 minutes after application, and the patch tests were read 48 hours after application.

Results

1. Inunction tests

The results of these tests are given in Table II. From this table it will be seen that all individuals previously recorded as sensitized in Experiment I showed unequivocal evidence of hypersensitivity of the skin of the previously exposed left forearm. This local hypersensitivity was evidenced in some instances by persistent erythema appearing within a few minutes after the inunction; and in all cases by an erythematous and edematous dermatitis at the 48-hour reading.

In 4 subjects of this Group A, sensitization could be shown to have affected also the skin of the not previously exposed right arm, as evidenced by varying degrees of dermatitis on this arm also. It is noteworthy that in 3 of these 4 subjects the reaction of the previously exposed left arm appeared earlier, and was also much more intense, than that of the not previously exposed right arm. In the 2 remaining subjects in Group A, the right arm showed no reaction to the inunction, although there was a reaction on the previously exposed left arm.

TABLE I

BAL and other compounds (dithiols) tested in Experiment II

BAL

Compound 2

Compound 3

Compound 4

Compound 5

Compound 6

Compound 7

TABLE II

Results of inunction tests and patch tests in Experiment II

Group	Volunteer	Vehicles used for BAL for sensitizing and/or control inunctions	14 daily applications of BAL 5 per cent on left forearm	Evidence of sensitization during or after 14 BAL applications	Inunction tests — Left arm Immediate reaction	Left arm Delayed 48-hr. reaction	Right arm Immediate reaction	Right arm Delayed 48-hr. reaction	Patch tests — BAL	Compound 2	Compound 3	Compound 4	Compound 5	Compound 6	Compound 7
A (exposed and showed clinical sensitization)	EPW	Grease oint.	yes	yes	(+)	+++	0	++	++	0	0	+-	+	(+)?	(+)?
	JTJ	Grease oint.	yes	yes	+	+++	0	+-	+-	~	(+)	++	-+	(+)	~
	DSG	Carbowax oint.	yes	yes	0	++	0	--	--	(++)	- 0	+	(+)	(+)	0
	HRA	Carbowax oint.	yes	yes	(+)	+++	+0	(+)	(+)	(++)	+	+	++	0	(+)
	CWZ	Ethyl. glycol	yes	yes	++	+++	+0	-+	-+	~	0	+	+-	0	0
	WVW	Ethyl. glycol	yes	yes		++	+	++	++	~	(+)	+	~	(+)	(+)
B (exposed but showed no clinical sensitization)	WEB	Grease oint.	yes	no	0	0	0	~	~	~	~	~	~	~	~
	RAS	Grease oint.	yes	no	0	0	0	(+)	(+)	~	0	0	0	0	0
	EWT	Carbowax oint.	yes	no	0	0	0	(++)	(++)	~	(+)	(+)	(+)	(+)	(+)
	ADR	Carbowax oint.	yes	no	0	0	0	~	~	0	0	0	0	~	0
	PNW	Ethyl. glycol	yes	no	0	0	0								
	RJS	Ethyl. glycol	yes	no	0	0	0	~	~	(+)	~	~	~	~	(+)
C ("fresh" subjects; not previously exposed)	JWH	Grease oint.	no		0	0	0	~	~	~	(~)	(~)	(~)	(~)	(~)
	WHE	Grease oint.	no		0	0	0	(+)	(+)	0	+	+	+	+	+
	TJF	Carbowax oint.	no		0	0	0	~	~	0	~	~	0	0	0
	RWS	Carbowax oint.	no		0	0	0	0	0	0	~	0	0	0	0
	BAD	Ethyl. glycol	no		0	0	0	0	0	0	~	0	0	0	0
	IPE	Ethyl. glycol	no		0	0	0	~	~	0	~	~	~	~	~
	DCF	Ethyl. glycol	no		0	0	0	~	~	0	~	~	~	~	~

The dermatitis produced by the inunctions in Group A was erythematous and edematous with some mottling and papule formation. Vesicular lesions, characteristic of the eczematous contact-type of allergic sensitization, were not observed.

In the subjects of Group B and Group C, there were no reactions to inunctions of BAL materials on either arm, i.e., no evidences of sensitization.

These results demonstrate that the 6 subjects in Group A still showed evidence of sensitization to BAL. In each of the 6 subjects, there were marked differences in the degree of sensitization produced at different skin sites. The *previously exposed skin sites* on the left arm *were on the whole significantly more sensitive* than the not previously exposed skin sites on the right arm; in 2 of the subjects, sensitization of the not previously exposed right arm was either absent, or so slight that it did not become clinically manifest under the conditions of these inunction tests. The significance of these results is indicated by the complete absence of reactions in Groups B and C.

2. Scratch tests

The scratch tests performed by the orthodox techniques produced variable degrees of erythema in different subjects. None of the compounds elicited greater response in Group A than in Group B and Group C. Moreover, there was no greater response to the scratch tests with BAL than to any of the other substances tested. Thus there was *no evidence of sensitization of urticarial type* demonstrable by this form of testing.

3. Patch tests

The results of the patch tests performed with orthodox techniques are included in Table II. Five of the 6 subjects in Group A gave definite reactions to BAL. The 1 subject in Group A who failed to give a definite reaction to BAL was 1 of the 2 subjects who had failed to react to the inunction test on the not previously exposed right arm. Obviously, in this subject the sensitization to BAL was even more localized than in the other 5 subjects of Group A.

In addition to the positive patch tests with BAL, 5 subjects in Group A gave definite reactions to compound 4, and 5 gave definite reactions to com-

pound 5. There were no significant reactions to either of these compounds in the subjects of Groups B and C. The conclusion therefore seems warranted that there were cross-reactions with compounds 4 and 5 in the subjects sensitized to BAL.

There was 1 significant reaction to compound 3 in Group A, and 2 significant reactions in Group B; and there was no reaction in Group C. This finding suggests the possibility, that there are cross-reactions to compound 3 in subjects sensitized to BAL. The single positive reaction to compound 1 is likewise insufficient to be regarded as evidence of cross-reaction. The patch test reactions clinically closely resembled the morphe of the reactions to inunction tests. They were all erythematous; or erythematous, papular and edematous; but never vesicular.

EXPERIMENT III—INCIDENCE OF SENSITIZATION TO BAL AFTER ITS APPLICATION ON DAMAGED SKIN

Subjects

Sixty-six subjects participated in this experiment.

Group A: 53 subjects who had been burned with mustard gas and who were known to have had previous exposures to BAL. The interval between first exposure to BAL and the time of application of the tests for sensitization varied from 9 to 33 days. In the 53 subjects of this group, the preceding applications of BAL were as follows:

In each subject an area on the flexor aspect of each forearm about 3.0×5.0 cm. to 6.0×8.0 cm. had been damaged by burning with liquid mustard gas. The center of the damaged area had sustained what was largely a third degree burn, but the peripheral areas were the site of either second and/or first degree burns.

Either 5 per cent BAL in grease ointment, 5 per cent BAL in carbowax ointment, 10 per cent BAL in petrolatum, or undiluted BAL had been applied 1 or more times to the damaged skin sites. In some of the subjects BAL had been applied to the damaged area on only *one* arm; in the remaining subjects, BAL had been applied to the damaged area on *both* arms.

Undiluted BAL was used in the form of a wet dressing left on for 48 hours. The BAL ointments were used as ointment dressings left on for periods varying in different volunteers from 48 hours to 7 days; or they were massaged into the damaged site 1 to 6 times at hourly intervals; or they were bandaged on following 1 application of a few drops of undiluted BAL.

Group B: 13 subjects who had no known previous exposure to BAL.

Procedure

Each subject received patch tests with 5 per cent BAL in grease ointment, and with this ointment vehicle alone (without BAL). The patch tests were applied to the grossly normal skin on the upper back. Furthermore, each subject received inunction tests with these two ointments. One-twentieth ml. of each ointment was applied to an area on the back, and then rubbed in for 30 seconds with a glass rod.

In those volunteers who had received applications of liquid BAL and/or BAL ointment only to the damaged site on one arm, the patch and inunction tests were carried out on the same side of the back, i.e. if a subject had had BAL applied to the left arm site only, both patch and inunction tests were done on the left side of the back.

Results

The results of this experiment are summarized in Table III. From this table it will be seen that among the 53 subjects in Group A, 35 (66 per cent) gave positive reactions to patch tests, and 27 (51 per cent) to inunction tests with BAL ointment. Among the 14 subjects who had been exposed previously on only one arm, 5 (36 per cent) gave positive reactions to patch tests; and 4 (29 per cent), to inunction tests with BAL ointment. Among the 39 subjects who had been exposed previously to BAL on both arms, 30 (77 per cent) gave positive reactions to patch tests; and 23 (59 per cent), to inunction tests with BAL ointment.

In none of the 13 subjects in the not previously exposed Group B were positive reactions elicited with BAL ointment in either patch or inunction tests. In none of the subjects of Groups A and B were positive reactions elicited by the ointment base alone in either patch or inunction tests.

EXPERIMENT IV—INCIDENCE OF SKIN SENSITIZATION TO BAL AFTER ITS PARENTERAL ADMINISTRATION

Subjects

Eighteen subjects participated in this experiment. These subjects had received 4 to 8 intramuscular injections of a solution of 10 per cent BAL and 20 per cent benzyl benzoate in peanut oil (6). The last injection of BAL had been given 13 to 25 days prior to the performance of the skin tests. The total dosage of BAL which had been administered intramuscularly varied from 930 mgm. to 2300 mgm. None of the subjects had had known skin exposure to BAL, except for the possible leaking of BAL solution onto the skin through the needle tract after the intramuscular injections.

Procedure

Each subject received patch tests with 5 per cent BAL in grease ointment, and 5 per cent BAL in carbowax ointment. The patch tests were applied to the grossly normal skin on the upper back. No control group of not previously exposed subjects was used in this experiment, since it had been shown previously, in other experiments, that the two BAL ointments employed produced no significant reaction when applied as patch tests to normal subjects not previously exposed to BAL.

Four of these subjects received a further intramuscular injection of 450 mgm. of BAL after the patch test reactions were read.

Results

Five of the 18 subjects showed evidence of skin sensitization to BAL in the form of positive reactions to the patch tests. Nine of the 18 subjects showed no reaction, while 4 showed reactions which were not sufficiently pronounced to be regarded as evidence of sensitization.

TABLE III

Incidence of sensitization to BAL after its application to damaged skin (Experiment III)

Total number of men.. 66
Group A (men previously exposed to BAL).. 53
Group B (men not previously exposed to BAL)... 13

	Inunction test positive		Patch test positive	
	Blank ointment base	BAL ointment	Blank ointment base	BAL ointment
53 men in Group A (previously exposed)	0 = 0 per cent	27 = 51 per cent	0 = 0 per cent	35 = 66 per cent
39 men in Group A (previously exposed on both arms)	0 = 0 per cent	23 = 59 per cent	0 = 0 per cent	30 = 77 per cent
14 men in Group A (previously exposed on one arm)	0 = 0 per cent	4 = 29 per cent	0 = 0 per cent	5 = 36 per cent
13 men not previously exposed	0 = 0 per cent	0 = 0 per cent	0 = 0 per cent	0 = 0 per cent

The clinical appearance, degree and intensity of the skin sensitization which resulted from the intramuscular injections of BAL, appeared to be similar to that observed in individuals who had become sensitized after repeated external applications of BAL to normal skin. It was noteworthy that the renewed intramuscular injection of 450 mgm. of BAL produced no cutaneous reaction or dermatitis in 4 of these subjects whose skin had become sensitized following the course of intramuscular injections of BAL given 15 to 27 days previously.

COMMENT

The results of these experiments clearly demonstrate some of the factors which determine the incidence of sensitization, as well as the level of sensitivity. We found an incidence of 19 per cent sensitization to BAL in individuals who applied BAL ointment or BAL solution to a site on one forearm daily for a total of 14 daily applications. This might be called the sensitizing capacity or sensitizing index of BAL on normal skin (8, 7). When BAL is applied repeatedly or for a prolonged period of time to a *damaged* area on one arm the sensitizing capacity of BAL is much higher, namely 36 per cent. After repeated or prolonged application to burned areas on both arms, the sensitizing capacity rose to 77 per cent. These results suggest that the incidence of sensitization is influenced not only by the number of applications and the concentration of the allergen used, but also by the size of the skin area exposed to the allergen, and perhaps also by the total quantity of allergen applied.

The finding that a contact-type sensitizing agent will produce a higher incidence of sensitization on inflamed or burned skin has previously been reported (9, 10). However, these tests with BAL are the first systematic experimental demonstration in human subjects of the difference in sensitizing capacity of an allergen when applied to grossly normal and to deliberately damaged skin. The most acceptable hypothesis for this increase in incidence of sensitization is that the presence of devitalized tissue at the site of application permits the ready combination of the smaller BAL molecules with proteins or other larger molecules, and thus accelerates the formation of complexes with a high capacity to sensitize. (For purposes of

comparison, the figures on incidence of sensitization are here given as percentages, despite the fact that the size of the series used may not be entirely satisfactory from a statistical viewpoint.)

These experiments demonstrated another interesting factor which has, to our knowledge, not previously been systematically studied in series of deliberately sensitized human subjects. We refer to the local differences in the degree of sensitivity at, or adjacent to, the site of sensitizing exposure, and at more distant skin areas. That there are very great local differences in the degree of sensitivity to contact-type allergens has been known for many years, since J. Jadassohn first called attention to the occurrence of such a phenomenon (11). It has more recently been discussed by Stauffer (12) and Sulzberger and Kerr (13).

Various degrees of spread (or lack of spread) of sensitization to BAL were observed. Thus in Experiment II all 6 subjects in Group A showed unequivocal evidence of sensitization on the previously exposed left forearm. In 2 of these 6 subjects, no evidence of sensitization could be demonstrated on the not previously exposed right forearm. In one of these 2 subjects, the sensitization appeared to be so localized that even a patch test with BAL on the upper back was negative. In 3 of the 6 subjects the not previously exposed forearm had become sensitized, but to a significantly weaker degree than the previously exposed forearm; while in 1 subject both forearms were equally sensitized.

Experiment IV showed that the skin can become hypersensitive to BAL subsequent to parenteral injection of this agent. It was further found that 4 subjects who had become skin hypersensitive to BAL (as demonstrated by positive patch tests), after repeated intramuscular administration, showed no cutaneous effects whatsoever when a large dose of BAL was again administered intramuscularly. Whether this phenomenon, which is obviously of considerable practical and theoretical significance, occurs in all cases of cutaneous sensitization to BAL cannot be decided on the basis of the 4 cases studied by us.

The type of skin sensitization produced by BAL as seen in our experiments is neither the classic eczematous contact-type (characteristic example: poison ivy sensitization) nor the classic urticarial type (characteristic example: sensitization to

"protein" fraction of ragweed pollen). In contrast to the usual forms of experimental contact-type eczematous sensitization, no vesicular reactions were seen either clinically nor in patch or inunction tests. Furthermore, there was a marked tendency for the BAL sensitivity to be significantly greater in the sites of actual previous exposure; and perhaps as a corollary to this, the dermatitis produced by BAL did not show the tendency to "spontaneous" spread or dissemination which is so commonly seen in many forms of eczematous sensitizations.

However, the BAL sensitization resembled contact-type eczematous sensitization in that the reactions were produced by external contact with the allergen, and the patch test reactions were positive after 24 to 48 hours, while the scratch tests for immediate wheal reactions were negative. In these last 2 respects, the BAL sensitization differs from the classic urticarial type of skin sensitization as seen, for example, in hayfever, asthma and atopic dermatitis; for in these the immediate wheal reaction is generally positive, and the response to the patch test is generally negative. The histologic examination of these reactions might have given further information on the type of sensitization produced. Unfortunately it was not possible to obtain biopsies from the reaction sites in our subjects.

The evidence at hand suggests that the sensitization produced to BAL is neither the classic contact-type of vesicular dermatitis, nor the classic urticarial form, but a somewhat different form of allergic response consisting of erythema, edema and papules upon external exposure of the skin to the allergen.

The cross reactions which BAL-sensitized subjects gave when tested with compounds 4 and 5 are interesting in that these 2 compounds are dithiol derivatives from which one would expect the original dithiol, i.e., BAL, to be liberated rather readily in the tissues.

SUMMARY AND CONCLUSIONS

1. When applied to normal undamaged skin, 5 per cent BAL in grease ointment, 5 per cent BAL in carbowax ointment, and 5 per cent BAL in ethylene glycol all produced definite sensitization and sensitization dermatitis in 16 out of 88 human subjects, i.e., in 19 per cent of the subjects exposed. There appeared to be no significant difference in the sensitizing capacity of BAL when used in these 3 vehicles.

2. When applied to damaged (chemically burned) skin, 5 per cent and 10 per cent BAL ointments and/or undiluted BAL produced definite sensitization and sensitization dermatitis in 35 out of 53 human subjects, i.e., in 66 per cent of the subjects exposed.

3. Although the series are too small to permit definite conclusions, our results suggest that there may be a significant difference in incidence of sensitization between those subjects who received BAL applications to the damaged (burned) site on only one arm (5 of 14 subjects, i.e., 36 per cent sensitized) and those who received BAL applications to burned sites on both arms (30 of 39 subjects, i.e., 77 per cent sensitized).

4. The above results may be explained by the fact that the presence of devitalized tissue at the site of application in burned area permits the ready combination of BAL with proteins, or other larger molecules, and the formation of complexes with a high capacity to sensitize.

5. The sensitivity produced by BAL was neither the classic eczematous contact-type nor the classic urticarial type of skin sensitivity. It differed somewhat from both of these common types and consisted of erythema, edema and papules upon external exposure of the skin to the allergen.

6. While the sensitivity to BAL did not remain strictly confined to the areas of exposure, there was evidence that in the majority of individuals the exposed area was more strongly sensitive than other parts of the skin. There was evidence that in exceptional cases the sensitivity may remain practically restricted to the previously exposed parts.

7. The sensitivity produced by BAL was not confined strictly to BAL, for the majority of sensitized individuals reacted also to patch tests with at least 2 chemically related compounds.

8. The repeated intramuscular injection of BAL produced skin sensitization as demonstrated by positive patch tests in 5 of 18 subjects. When a large dose of BAL was again injected intramuscularly, in 4 of these subjects whose skin had become hypersensitive to BAL, no cutaneous reactions were produced.

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CLINICAL USES OF 2,3-DIMERCAPTOPROPANOL (BAL). VI. THE TREATMENT OF COMPLICATIONS OF ARSENO-THERAPY WITH BAL (BRITISH ANTI-LEWISITE) [1]

By A. B. CARLETON, R. A. PETERS, L. A. STOCKEN, R. H. S. THOMPSON, AND D. I. WILLIAMS WITH APPENDIX UPON EXCRETION OF ARSENIC
BY I. D. E. STOREY, G. A. LEVVY, AND A. C. CHANCE

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INTRODUCTION

This report presents the clinical evidence available in Great Britian up to September, 1944, upon the therapeutic effect of British Anti-Lewisite or BAL (also called OX 217 for security reasons during the war) in cases of arsenical dermatitis. There is some indication that this compound has a curative action; but as often happens in clinical trials, the evidence collected is not yet sufficient to present a definite case, even after a year's work, owing to the scarcity of available material. Nevertheless, it is certain that the compound merits further trial. A brief summary will be given of the biochemical background and of the clearcut experiments on which promise of clinical success is based, as well as a discussion of what can be expected clinically from an arsenical antidote.

In recent years, as the results of research in many laboratories, progress in our knowledge of the intermediary metabolism of carbohydrates has led to more detailed information on the progressive steps by which the carbohydrates, glucose and glycogen, are broken down in cells to carbon dioxide and water; a recent review of this work is given by Meyerhof (1). Each step is controlled by a definite enzyme, and many involve phosphorylations. One of the penultimate stages of this degradation is pyruvic acid, a three carbon keto-acid. In so far as tissue cells rely upon carbohydrates for their energy, the stages in breakdown are all of importance. It follows that one way of interfering with cell life is to interrupt the activity of some enzyme essential to tissue carbohydrate metabolism. For instance, it is known that when thiamine (aneurin) is deficient in brain tissue, the brain cells concerned are sufficiently disturbed to produce a convulsive state in the animal: here aneurin is a component of the organized group of enzymes (or 'enzyme system') responsible for the oxidation of pyruvic acid, which is usually called the pyruvate oxidase system. This system has protein and other components; hence interruption of tissue metabolism can occur at this stage not only by deficiency of the vitamin, but also by active interference with the functioning of any of these components by poisons. This work has been reviewed by Peters (2) who has generalized such changes as 'biochemical lesion.'

Some years ago it was realised by Peters (3) that the poisons, iodoacitic acid and dichlordiethyl sulphone, which can induce pathological change in skin, have a selective inhibitory action on the pyruvate oxidase enzyme system. It was further known before the war that arsenite too interferes with the metabolism of pyruvic acid (4) and that the pyruvate oxidase system contains a component sensitive to very small concentrations of arsenite, which also act selectively on it at these levels (5, 6). It follows that the metabolism of carbohydrate is poisoned at an important stage by traces of an arsenical. This is the predominant way in which arsenicals can cause biochemical lesions, and any antidote must be capable of reversing this biochemical damage.

On the basis of these facts, it is logical to use the pyruvate oxidase system as well as the more classical method of in vivo injection as a test for new antidotes against arsenic, and during the war a research upon these lines was initiated and

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pursued by a team in Oxford (7). As a result of the systematic attack made on this problem from the biochemical angle, it was found (8, 9) that simple 1,2-dithiols (of which BAL,$CH_2SH \cdot CH$-$SH \cdot CH_2OH$, is an example) are capable both of exerting a marked antidotal action against the poisoning of this enzyme system by trivalent arsenicals, and, more important still, of reversing this poisoning when once established. These effects are brought about through the ability of 1,2-dithiols to form stable ring compounds with trivalent arsenicals,

$$
\begin{array}{l}
CH_2SH \\
| \\
CHSH \qquad +OAsR \rightarrow \\
| \\
CH_2OH
\end{array}
\qquad
\begin{array}{l}
CH_2S \\
| \quad\diagdown \\
| \qquad AsR \qquad +H_2O \\
CH_2S \diagup \\
| \\
CH_2OH
\end{array}
$$
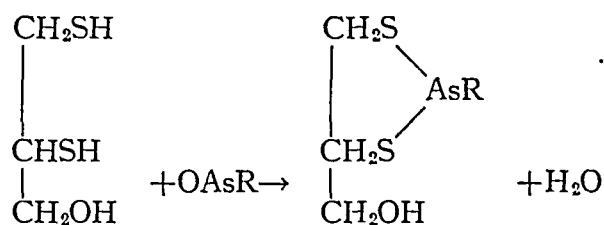
instead of the much more dissociable "open chain" compounds formed between arsenic and the monothiols investigated in the past.

It was at once clear that in addition to experiments with Lewisite, extended *in vivo* trials with the therapeutic arsenicals were needed. These were undertaken and it was clearly shown that the administration of BAL is highly effective in bringing about the survival of rats after the injection of lethal amounts of Mapharside (M-amino-p-hyrdoxyphenylarsenoxide hydrochloride), even when the drug is not administered until after the injection of a lethal dose of the arsenical. The results of some experiments reported elsewhere (10) given in Table I will illustrate this:

Mapharside, freshly dissolved in glass-distilled H_2O, was injected intramuscularly into the hind-legs of rats weighing from about 150 to 200 grams each. At intervals varying from 5 to 15 minutes after injecting the arsenical, BAL, dissolved in propylene glycol, was injected intramuscularly in doses of 40 to 50 mgm. per kgm. into the opposite hind leg.

It should be pointed out that although the interval between injection of the arsenical and treatment with BAL is short, the toxic effects produced by doses of Mapharside of this order come on with great rapidity: after 15 minutes the rats already show marked signs of generalized intoxication (salivation, lachrymation and diarrhea), many being in a state of collapse. Similar results

TABLE I

Effect of injected BAL on the survival of rats after administration of lethal doses of Mapharside

	Number of rats	Maphar-side	Interval before treatment	Survivors	
				Number	Per cent
		mgm. per kgm.	min.		
Controls	4	18 to 19		1	25
	14	28 to 32		3	21
	4	43 to 44		0	0
	6	45 to 52		1	17
	6	42 to 45		0	0
Treated	6	30 to 33	15	4	66
	6	30 to 32	7	6	100
	5	37 to 40	7	5	100
	6	43 to 47	5	6	100

while this work was in progress were reported from America by Eagle (11).

Biochemical evidence has therefore been obtained which shows that BAL is capable not only of combining with free, circulating arsenic, but also of displacing it once it has reacted with tissue components. In view of this, it was expected that BAL therapy might lead to an increased renal excretion of arsenic. This point has been studied in animals suffering from poisoning by trivalent arsenicals, and it was clearly shown by Thompson and Stocken (12) that under the conditions of these experiments, treatment with BAL brings about a significant and rapid increase in the amount of arsenic excreted in the urine. More recently results from Edinburgh strikingly confirm this (13).

The experimental work outlined above was so definite that it justified a study of the efficiency of this compound in the treatment of toxic manifestations of the therapeutic arsenicals in man. For the initial assessment, it seemed desirable to investigate severe cases showing signs of sufficient intensity to reveal any specific therapeutic action, and it was therefore decided to begin with a study of BAL therapy in cases of acute exfoliative arsenical dermatitis.

METHOD OF ADMINISTRATION

In view of the fact that BAL had not previously been administered internally to man, it was decided to commence with inunction of a 5 per cent ointment.

Applied by this route and in this concentration, it was known from earlier work in Oxford and in the U. S. A. that the compound is rapidly absorbed, and that the oint-

ment can be applied over large areas of normal skin without giving rise to any severe systemic toxic effects. Locally the application of BAL to the skin does bring about transient erythema and tingling, and in some persons, an edematous, urticarial reaction, which subsides, however, in about 2 to 3 hours. Repeated application of BAL to the same area of skin is to be avoided on account of the possibility of a low-grade skin sensitization to the compound, as demonstrated by Sulzberger and Baer (14). It seemed likely that application to an already inflamed skin should also be avoided, so that in the treatment of cases of dermatitis the inunction was made into normal skin, or, in cases where the skin involvement was general, into an area of skin showing minimal involvement. In all except the earliest of the cases treated by inunction, applications of approximately 1 gram of the ointment were rubbed in over a small area of skin, and when repeated on successive days, different areas were chosen each day.

The BAL was made up on a 5 per cent W/V basis in the following vehicle:

Lanoline ....6 grams
Lanette wax ....4 grams
Diethyl phthalate ....2 grams

It was soon realized that although some success was attending this method of administration, the route was far from satisfactory, and it was decided to turn to injection.

At the time when we were considering, as a first step, the injection of aqueous solutions of BAL, known to be effective in animals (15), our attention was drawn (priviate communication) to Eagle's demonstration (16, 11) that 5 per cent BAL in 10 per cent benzyl benzoate in peanut oil, given by intramuscular injection, is an effective therapeutic agent in animals, and that this solution was also suitable for administration to man. As there was some urgency in arriving at a suitable method of administration, we decided at once to try Eagle's formula. An improved method of ampoule preparation (17) ensured that less than 1.5 per cent of BAL was destroyed during the sterilization, as compared to 10 per cent by Eagle's original method. When adapted for large-scale production, the final ampoules contained a 5 per cent W/V solution of BAL in 10 per cent W/V benzyl benzoate in peanut oil, being nitrogen-filled and sterilized by heating for 1 hour at 170° C.

The total dosage given varied from case to case according to the condition of the patient, but usually consisted of a course of 2 ml. given by deep intramuscular injection into the gluteal region twice daily for 3 or 4 days, followed by a further course, if no obvious improvement occurred, or if, at any time, there were signs of any relapse in the condition of the skin.

In the great majority of cases the injections have been well tolerated, but in a few, local abscesses developed at the injection sites. This point will be discussed later in greater detail, but the impression gained was that the abscess formation was as much due to the general septic condition of the patient, arising from the heavy secondary infection of the skin not infrequently present, as to the BAL itself.

Considerations upon skin conditions due to arsenicals.

Certain changes may be expected in the skin of patients developing an arsenical dermatitis.

(a) Very small amounts of arsenic will be enough to cause biochemical lesions, or even death, of the tissue cells. For instance, it can be calculated that an area of skin 30 cm. square, might be poisoned by as little as 0.02 mgm. arsenic. Even if, therefore, an antidote removes arsenic by combination with it and excretion of the resulting product, the increase in the variable daily excretion of arsenic in the urine might be too small to be readily detected.

(b) Cells subjected to the action of arsenic for some time may be able to live feebly by relying upon other channels of metabolism, and will not immediately recover completely when the combined arsenic is removed. Hence a pathological state may remain as long as the slow rebuilding of cell enzymes takes place. If there is, in addition, much loss of plasma protein by weeping, and of skin by exfoliation, this will be a cause for additional delay in healing; there will, in effect, exist a condition analogous to that of a thermal burn.

(c) It is almost certain that tissue stores of arsenical compounds must exist in quantities such that, even when arsenic has been removed from the skin, the weakened cells may be repoisoned, occasioning relapses. This condition would be more likely to occur in cases subjected to long periods of arsenical treatment.

(d) Toxic manifestations are not necessarily due to the actual presence of arsenic, but may result from sensitization to a foreign protein brought into being by the arsenic. In these cases, there may be no arsenic in the skin, so that the dermatitis would not be affected by an arsenical antidote.

Reflection upon these 4 points indicates that in all probability, some cases of so-called arsenical dermatitis cannot respond to an antidote, and, further, that in those cases that do respond the signs due to the immediate presence of the arsenic will disappear, but will leave a slowly healing residuum. The clinical proof is further handicapped by the additional complication of skin in-

fection. We should expect the most direct prooofs of therapeutic value in acute' cases resulting from intensive therapy with Mapharside, since they will have the highest body content of arsenic at the time of dosing.

RESULTS

The 30 cases described in this article may be grouped into:

(a)   18 cases treated by injection.
(b)   9 cases treated by inunction.
(c)   3 cases whose interpretation, for reasons described later, is uncertain.

Detailed clinical notes of the cases, together with a summary, are given in Appendix I, and the results of arsenic estimations in the urine and skin in Appendix II.

In order to simplify the interpretation of the progress observed, all other local treatment was, whenever possible, discouraged, and to no case after coming under our notice were preparations of sodium thiosulphate given. Where circumstances permitted, the skin was photographed before starting treatment with BAL, and again as soon as any obvious improvement had taken place.

(a) Cases treated by injection.

The 18 cases in this series were all severe or moderately severe, showing weeping or desquama-

tion or exfoliation from the affected areas. Two of the cases ended fatally: Case No. 6 died suddenly after mild rigors, and at autopsy showed a terminal broncho-pneumonia, while Case No. 15 developed a sloughing glossitis, stomatitis and pharyngitis and an early granulocytopenia.

The duration, in days, of the skin disturbance in the remaining 16 cases is given in Table II. It will be seen that the mean duration is 38.9 days, with a range of from 5 to 90 days. In 6 of the cases, the patient was not followed through until the skin was reported as normal; Table II, therefore, also gives the dates when the skin was reported as 'almost normal,' i.e., normal except for some slight and localised disturbance, the nature and extent of which can be seen by reference to the case report.

Of the 18 cases treated by injection the number of days from the first injection of BAL to the time when the skin became 'normal' or 'almost normal' is also given, the mean times being 27.6 days for those followed through to 'normal' and 18.9 days for those in which an 'almost normal' report was received.

It will be seen from Table II that 3 of these injection cases (Nos. 8, 11 and 18) showed no dramatic response to the drug as regards the time taken for complete, or nearly complete, heal-

TABLE II

Duration of dermatitis in patients treated by injection

Case number	Date of onset	Date of first injection of BAL	Date when 'almost normal'	Date when normal	Total duration of dermatitis	Number of days from first injection to time when	
						Normal	'Almost normal'
					days		
1	10.10.43	3.11.43	3.12.43		54		30
2	7.10.43	12.11.43	3.12.43	7.12.43	61	25	21
3	21.11.43	5.12.43		14.12.43	23	9	
4	2.12.43	8.12.43		14.12.43	12	6	
5	22.12.43	26.12.43	5. 1.44	14. 1.44	23	19	10
6	Died						
7	23.12.43	9. 1.44	18. 1.44		26		9
8	31.12.43	26. 1.44	2. 3.44	11. 3.44	72	45	36
9	2. 2.44	5. 2.44	12. 2.44	4. 3.44	31	28	7
10	3. 2.44	14. 2.44	28. 2.44		25		14
11	26. 1.44	16. 2.44	6. 3.44	25. 4.44	90	69	19
12	24. 2.44	18. 3.44	22. 4.44		58		35
13	18. 2.44	29. 2.44	14. 3.44	23. 3.44	34	23	14
14	18. 2.44	6. 3.44		30. 3.44	41	24	
15	Died						
16	24. 2.44	6. 3.44	9. 3.44		14		3
17	Dates not given				5		3
18	20. 4.44	29. 4.44	13. 6.44		54		45
				Mean =	38.9	27.6	18.9

ing of the skin, Cases No. 8 and 11 taking 45 and 69 days respectively to return to normal, and Case No. 18, 45 days to return to 'almost normal.'

As pointed out earlier in this report, however, it is unlikely that the skin in all cases of arsenical dermatitis will heal rapidly following treatment with an arsenical antidote unless other measures are also taken to combat the loss of protein that is probably occurring simultaneously, owing to weeping or desquamation. It is of interest therefore to inspect the case reports in order to discover whether any partial healing, such as cessation of oozing or exfoliation, regularly took place after the injection of BAL, even though some residual skin changes, such as a persisting erythema, might remain.

In Table III are given the reports of such progress in the various cases at the stated number of

TABLE III

Progress of cases in response to BAL injection

Case number	Reported Progress	Number of days after first injection
1	All weeping ceased except for very slight patches at upper end of sternum and on buttocks	6
2*	Definite improvement.	2
	Natural elasticity of skin almost regained.	6
3	Skin normal.	9
4	Rash disappeared. Residual pigmentation present.	6
5	Skin normal except for soles of feet.	10
6	Died.	
7	Skin clear of all scaling and erythema except for scalp and feet. (Result complicated by report of improvement 2 days prior to injection.)	9
8	All skin areas dry. Still some desquamation on neck, abdomen and forearms.	16
9	Skin normal except for slight erythema of chest and desquamation of palms and soles.	7
10	Marked improvement. No edema. Skin dry and returning to normal color. Scattered scaling, particularly on scalp.	14
11	General improvement. Still some dry desquamation with generalized erythema.	19
12	Condition satisfactory. Skin improving steadily.	12
13	General condition much improved. Skin dry and erythema subsiding.	10
14	Marked improvement. Hands and feet stopped weeping.	14
15	Much less edema.	4
	Relapse. Died.	
16	Skin practically normal.	3
17	Skin practically normal.	3
18	Skin slowly returning to normal.	45

* Local applications of BAL ointment given one month previously.

days after the first injection of BAL. It will be seen that in all except the last case (No. 18), improvement, and in many instances marked improvement or a complete return to normal, took place in under 3 weeks from the first injection.

It must be pointed out that in only 4 of these cases was the dermatitis uncomplicated at any stage by other lesions, usually infective in type. Table IV briefly summarizes both the local com-

TABLE IV

Complications present in cases treated by injection

Case number	Skin	General
2	Secondary infection of toes and scalp.	Purulent conjunctivitis upper respiratory infection.
4		Jaundice.
5		Abscess Rt. and Lt. gluteal region.
6		Terminal bronchopneumonia.
7	Generalized secondary infection of skin.	Conjunctivitis. Blepharitis. Multiple styes and boils.
8	Severe staphylococcal infection.	Axillary and inguinal adenitis.
9		Jaundice.
10	Secondary infection of forearms.	
11		Abscess Rt. and Lt. buttock.
12		Infected throat. Abscesses in axillae and pubic region.
13	Infected patches on each cheek.	Abscess Lt. buttock
14	Generalized secondary infection.	
15		Purulent conjunctivitis. Sore throat. Early granulocytopenia.
16	Late pyoderma	
18		Slight conjunctivitis and ulcerative stomatitis.

plications occurring in the skin, such as secondary pyogenic infection, and any other complications occurring either generally or in other organs.

Table V shows the incidence of local reactions to the injections in all cases, i.e., in the 18 cases of the series and in the 3 anomalous cases discussed later. It will be noted that in 4 patients, gluteal abscesses developed at the injection sites, while in a fifth, slight heat, tenderness and stiffness of the buttock appeared in 6 hours, but disappeared completely in 24 hours. In the remaining 16 cases, no local reaction was reported, though a few of the patients experienced a generalized irritation

TABLE V

Incidence of local reactions to injection of BAL

Case number	Local reaction
4	Slight heat, tenderness and stiffness of the buttock, appearing in 6 hours, and disappearing in 24 hours.
5	Bilateral gluteal abscesses.
11	Bilateral gluteal abscesses.
13	Abscess Lt. buttock.
28	Two abscesses at injection sites.

No reaction in 13 of the 21 cases.

of the skin without visible signs following the injections.

(b) *Cases treated by inunction.*

Of these, the earliest cases studied, 4 out of 9 (Nos. 19, 20, 26 and 27) were comparatively mild, and never progressed to the stages of weeping or widespread desquamation, thus making it difficult to decide whether the progress observed was a true consequence of the BAL inunctions.

(c) *Remaining cases.*

Three further cases (Nos. 28, 29 and 30) are included, although the interpretation of therapeutic effects in them was very uncertain. In Case No. 28 it was impossible, owing to the existing war conditions, to obtain sufficient evidence of the patient's progress, particularly in the earlier stages; the patient received only 1 course of injections, and it is not clear from the records at our disposal whether a second course might not have been of use. In Case No. 29, although a marked improvement was noticed in the condition of the skin 2 days after the first injection, the patient relapsed one week later and was not given any further treatment. In Case No. 30 the eruption, though generalized, was so slight as to render difficult the interpretation of the rapid improvement observed.

DISCUSSION

The earliest reported work which is known to us on the treatment of arsenical dermatitis with BAL is that of Longcope, Wintrobe and Luetscher (18). They treated 6 patients, suffering from phenarsazine chloride dermatitis, by inunction of the affected areas with a 5 per cent BAL ointment. The results were described as good; the ointment

was also effective when applied to the normal skin. Somewhat later the same authors stated that such treatment was followed by an increased excretion of arsenic in the urine. This account makes mention of 2 other patients treated by inunction, 1 had agranulocytosis and the other generalised exfoliative dermatitis, due to injected mapharsen and neoarsphenamine. Later, Eagle (16) reported a series of cases of arsenical dermatitis treated with injections of BAL; he suggested that there was evidence of a beneficial effect both upon the dermatitis and upon jaundice, when present. Our experience of treatment of post-arsphenamine jaundice with BAL was not favourable. In a series of cases treated at Netley by inunction there was no evidence that the jaundice was improved; in fact, most cases of so-called post-arsphenamine jaundice do not appear to be due to the continued presence of signficant quantities of arsenic in the liver (19).

The majority of cases discussed in this report had been treated with neoarsphenamine. The excessive reaction to small doses of the arsenical suggests that some of these cases are associated with some degree of arsenic sensitisation; Case No. 5 is a good exmaple of this. As will be seen in Table II, some cases showed a much quicker cure than others.

Although more patients are needed to get an adequate assessment of the clinical value of BAL, we shall attempt a summary of the position. The success of the new therapy must be judged by the acceleration in the rate of cure as compared with similar cases not treated with BAL. We have had the greatest difficulty in arriving at the average duration of severe cases of arsenical dermatitis treated by methods other than BAL. From notes made by one of us (D. I. W.) at Netley, we have a mean time of cure for 5 severe cases of 55 days (28 to 102). Two other cases of which we have received notes from Major Bolton lasted for 60 and 118 days. Davies (20) records the average duration of cure for 135 cases at his hospital from 1929 to 1941, who had been dosed with neoarsphenamine, as 62.5 days, calculated from weighted mean figures. Mannix (private communication) considers 46 days an average time for cure for cases of varying severity. It is a well established clinical impression that patients showing exfoliation and oozing may take more than 2 months to

cure. If we accept these estimates as the best available at present, it will be seen that the mean duration of 38.9 days (Table II) for our cases treated with BAL is a definite improvement. By a rough clinical assessment of each case, the conclusion was reached that 1 in 2 cases had been markedly improved by the treatment. Case No. 2 seems especially pertinent, because only a temporary improvement was made by inunction, whereas about 1 month later, injection caused a dramatic response. This does not present such a striking picture as that claimed in the early U. S. reports (21, 11, 16); but from the clinical data in these reports it is difficult to discover the time for complete recovery in several cases, making comparison with our assessment difficult.

There seems to be a definite tendency in more than 1 case for a rapid cessation of the oozing, followed by a more intractable persistence of the erythema. This happened for instance in Case No. 1, where the erythema could not be reduced by inunction of an erythematous patch with BAL. This is consistent with the idea that capillary damage may be associated especially with the continued presence of arsenic, but that when this is removed, some damage to blood vessels remains and causes a slowly fading erythema. The low blood protein values suggest that general measures should be taken to combat loss due to exfoliative conditions; a condition of protein deficiency alone would be sufficient to delay healing.

The assumption has been made throughout this report that the curative agent in the ampoules is BAL itself; on experimental grounds this assumption is justified. Nevertheless, we had planned to study, as a control, the effect of ampoules containing only peanut oil and benzyl benzoate; the opportunity for this has not yet arisen.

Reference to Appendix II shows that there was no experimental evidence of increase in arsenical excretion as a result of injecting BAL. In our opinion, close examination of the data in Eagle's report does not bear out his contention that arsenical excretion was increased. We are therefore forced to conclude that no such effect has been clearly demonstrated in cases of this type. At the same time, we think that this result is not surprising. It is known that damage by an arsenical is induced with very small amounts of the poison, and that the metabolically active layers of epidermis are not deep. The arsenic content of the urine is low; since, therefore, the arsenic responsible for continued damage in the skin is probably no more than a few micrograms, this would be masked by the general day-to-day fluctuation in arsenic excretion. In our patients, many of the figures approach normal levels. Bang (22) recorded values of 0.01 to 0.06 mgm. arsenic per L. as usual values in the urine of normal individuals, though occasional excretions of 0.2, 0.4 and even 0.69 were noticed; these marked daily excretions in the same individuals probably depend upon the arsenic content of the diets. Webster (23) found average values of 0.015 mgm. per L. for a group of normal individuals, and rather higher values, 0.050 mgm. per L., for those who had ingested fruit sprayed with lead arsenate. The late Professor A. J. Clark considered values for arsenic in urine in mgm. per day as follows: 0.1 mgm. or less physiological; 0.1 to 1.0 mgm., doubtful; and 1.0 mgm. or more, direct poisoning. Our cases mostly fall in the doubtful class.

In confirmation of the results upon our patients, Chance (13) reported that injection of BAL did not increase urinary arsenic excretion in a rabbit with low total arsenic in the urine following dosage with Mapharside. It was quite otherwise when they had recently injected an arsenical, and there is some evidence by Black & Trinder (private communication), to be published later, which suggests that BAL does increase excretion of arsenic in patients recently dosed with an arsenical drug.

Several cases also showed evidence of relapse. This can be explained if 'arsenic' from some other part of the body enters the blood stream and again poisons the skin. From our experience, good results have followed a second course of injection of BAL.

We have also had the opportunity of treating 2 cases of agranulocytopenia, and 1 of encephelopathy through the kindness of Lt. Col. Gordon and Major Blaisdell, R.C.A.M.C., and the Medical Officers concerned; three cases of agranulocytosis have been treated with BAL under the direction of Lt. Col. Pillsbury, U.S.A. Medical Corps. Although recovery has taken place in each of these cases, it is clear that we can conclude nothing final until more cases have been studied. It will be noted also that the excretion of arsenic was not much increased in Col. Gordon's 2 cases.

It is realized that the number of cases available for this report is too small to eliminate the possibility of serious statistical error in the interpretation of the results. On the other hand, the striking clinical effect in some of the cases, and the apparent reduction in the time of healing, warrants a more extended trial of this new compound. The experimental evidence obtained from animals further supports this contention.

SUMMARY

1. Experimental evidence has shown that BAL will reverse the toxic action of Mapharside in animals.

2. BAL has been administered to 30 patients suffering from arsenical dermatitis, mostly following injections of neoarsphenamine; 21 of these cases were treated by intramuscular injection; and the remainder, by inunction. The clinical evidence indicated a beneficial effect in a substantial number of cases.

3. Estimation of arsenic excreted in the urine of several patients did not reveal any significant change due to treatment by BAL. The interpretation of this is discussed.

Case No. 1. Male, aged 30.   Radcliffe Infirmary, Oxford.
Date   *Professor L. Findlay.*

14.7.43 to 15.9.43. N.A.B. 4.15 grams. Bis. 2.1 grams.
22.7.43. Pot. iodid. mixture.
15.10.43. Seen with rash on trunks and limbs, saying it had started 5 to 7 days previously. Skin itchy.
20.10.43. Admitted to hospital. Thiostab. 0.6 gram intravenously b.d. for 5 days, and then on alternate days.
30.10.43. Transferred to Radcliffe Infirmary, Oxford.

APPENDIX I

The treatment of complications of arseno-therapy with British Anti-Lewisite containing Summaries and Case Histories

SUMMARY OF INJECTION CASES

Case number	Condition at commencement of treatment	Total number of days* on which BAL was injected	Final report on condition of case	Number of days from first injection of BAL
1	Severe generalized dermatitis with much crusting and scaling.	11	Skin condition practically normal, except for slight erythema over inner side of lower thighs.	30
2**	Severe generalized dermatitis. Large areas on trunk and legs moist and desquamating.	6	Skin normal.	25
3	Generalized erythroderma. Fine desquamation on face. Dry, branny rash on trunk and arms. Coarser exfoliation on legs.	2	Skin normal.	9
4	Generalized rather coarse eruption, characterized by irregularly-shaped pink splashes, dry and rough, and fine papules or small flat irregular plaques with whitish surface.	3	Rash disappeared. Residual pigmentation present.	6
5	Generalized exfoliative dermatitis.	3	Skin normal.	19
6	Severe generalized exfoliative dermatitis.	3	Died.	
7	Generalized moderately severe exfoliative dermatitis.	3	Skin clear of all scaling and erythema except for scalp and feet.	9
8	Generalized exfoliative dermatitis affecting the whole body except the soles of the feet. Oozing of scalp and inner side of forearms, thighs and legs. Exfoliating elsewhere.	12	Skin normal.	45
9	Generalized dusky erythroderma of whole body, with fine desquamation, tending to be coarser on the face. Erythema on chest.	6	Skin normal.	28
10	Generalized dermatitis with desquamation and weeping.	3	Skin dry and returning to normal color. Scattered scaling, particularly on scalp.	14

APPENDIX I—*Continued*

Case number	Condition at commencement of treatment	Total number of days* on which BAL was injected	Final report on condition of case	Number of days from first injection of BAL
11	Generalized angry erythema. Extensive weeping over trunk and limbs. Exfoliation beginning over shoulders, inner side of arms, thighs and creases of abdomen; face and scalp clear.	4	Skin normal.	69
12	Exfoliative dermatitis, generalized except for hands and feet.	6	Skin satisfactory except on face and hands which are still very dry and scaly.	35
13	Erythema with commencing desquamation over whole body. Exfoliation over lower third of both legs.	4⁻	Skin normal.	23
14	Generalized dusky red morbilliform rash with yellowish powdery desquamation. Face swollen and coarsely desquamating. Hands and feet peeling and weeping.	6	All traces of dermatitis vanished.	24
15	Generalized erythema and edema. Scales on face, neck and ears. Serous discharge from face. Extensor surfaces of forearms and thighs dry and scaly.	8	Died 17 days after start of treatment (skin reported much improved 12 days after start of treatment).	
16	Severe, diffuse weeping dermatitis.	4	Skin practically normal in appearance.	3
17	Generalized erythema with considerable itching. Some exfoliation.	2	Skin practically normal.	3
18	Vesicular rash on arms and legs with edema and crusting of exudate. Slight ulcerative stomatitis.	7	Skin slowly returning to normal. Dry and scaly all over.	45

* In several cases the injections were given in divided courses. For details see case reports.
** Local applications of BAL ointment given one month previously.

Condition on admission: Temp. 100°. Generalized dermatitis with much desquamation. Rash severe on abdomen, thighs and backs of legs. Back and face less severely affected; buttocks, feet, skin over patellae quite free.

2.11.43. More weeping of skin of thighs (front and back). Rest of body rough, with much crusting and desquamation. Penis edematous; elsewhere no edema. Temp. 100.2°, white count, 10,000, eos. 14 per cent.

3.11.43. Temperature normal for first time since admission. Patient slept better. Skin around neck, in groins and inside of thighs definitely more inflamed; much desquamation. Face today, including ears, more involved. Tongue furred but moist. BAL 1 ml. injected into gluteal region.

4.11.43. Slight itchiness in back but slept well. Skin generally much improved. Less erythema and less oozing in groins and thighs. Back drying up. BAL 1.9 ml. given.

5.11.43. Skin still further improved. Erythema less and now limited to groins, scrotum and hypogastrium. Less crusting, but still considerable dry

desquamation. Tongue cleaner. Appetite much improved during last 24 hours. BAL 2.0 ml.

6 and 7.11.43. No injections.

8.11.43. Much desquamation of skin generally, and hyperaemia of newly exposed dermis. Still complains of irritation, especially around shoulders. BAL 4.0 ml.

9.11.43. No weeping, except for very slight patches at upper end of sternum and on buttocks. Desquamation. Skin generally softer. BAL 2.0 ml.

10.11.43. Definite improvement: erythema more patchy with healthy skin between. Greatest improvement in thighs. Skin generally dry with finer desquamation, but some moisture still present in popliteal spaces and inside knees. BAL 2.0 ml.

11 and 12.11.43. No injections.

12.11.43. Condition further improved. Skin more healthy. Patches of erythema and thickening less. Less swelling and desquamation on face and ears. Itch seems less marked and patient looks more comfortable. No weeping anywhere to-day.

13.11.43. BAL 2.0 ml.

14.11.43. Skin slightly better. Some irritation at upper

CASE No. 1

3rd November 1943.

8th November 1943.

end of sternum and on inner sides of thighs and popliteal spaces. BAL 2.0 ml.

15.11.43. BAL 2.0 ml.

16 and 17.11.43. No injections.

16.11.43. Skin unchanged. BAL ointment applied to patches of erythema below right nipple; irritation for 2 hours after application, but no certain change visible on 17.11.43.

18.11.43. Skin condition unchanged. BAL injections, morning 1.8 ml., evening 2.0 ml.

19.11.43. Since first few days after treatment started, the improvement has been gradual, and there has been no relapse. Erythema has not yet disappeared on body or limbs. No tendency to weeping except in popliteal spaces. BAL injections, 2.0 ml. morning and evening.

25.11.43. Skin much improved.

26.11.43. Erythema around neck and in popliteal spaces much improved.

1.12.43. Very marked improvement in skin. Erythema present only on inside of thighs and lateral aspects of chest.

3.12.43. Skin condition practically well. With exception of slight erythema on inner side of lower thighs, no dermatitis visible (Aspirin gr. x).

General comments: There has never been any irritation or pain or tenderness at the site of injection. No ointments were used. Previous to 3.11.43 light diet given, but containing some meat, chicken, fish and eggs.

From 25.11.43 to 30.11.43 given casein biscuits (=60 grams casein per day) in an attempt to accelerate healing.

Note 1 by Professor Findlay. This patient seemed to make good recovery at first, but latterly improvement was slow and complete recovery was not more rapid than has occurred in equally severe cases treated with sodium thiosulphate intravenously and coal tar locally.

It is interesting to note the eosinophilia, seen also in other cases, and the fact that investigation of hepatic efficiency showed no evidence of liver damage.

Note by R. A. P. This case was seen throughout by R. A. P., and further estimations in addition to those mentioned in Note 1 were done in the Nuffield Laboratory of Biochemistry.

Blood urea. 2.11.43. 43 mgm. per 100 ml.
12.11.43. 28 mgm. per 100 ml.
22.11.43. 32 mgm. per 100 ml.
3.12.43. 28 mgm. per 100 ml.

The rather high value was therefore reduced after treatment.

Blood proteins	Total grams per cent	Albumen grams per cent	Globulin grams per cent
2.11.43.	5.2	3.2	1.55
12.11.43.	6.5	3.7	
22.11.43.	6.34	3.7	
3.12.43.	6.2	3.6	

The blood proteins were low, though improving during treatment. Evidently cases showing much weeping should be treated like "burn" cases; there is a distinct indication for the administration of high protein diets.

Urinary creatine, creatinine and total nitrogen contents were determined daily with the technical assistance of R. W. Wakelin. Before treatment, urinary creatinine values were high, and creatine values of 1.2 grams per diem were found; by the start of the treatment however these abnormalities had disappeared. However, throughout the period in hospital, the total nitrogen content of the urine was high; unfortunately, owing to war-time conditions, it was not possible to get a proper estimate of food intake, but urinary N values of 20 grams and over, were recorded on several days, equivalent to protein values of 125 grams and more. There is again a suggestion here that negative balances occur in these patients (as in burns), a question which should be investigated when conditions are more favourable.

Photographs appended.

Case No. 2. Male, aged 28.   R. N. Hospital, Chatham.
        Surgeon Rear-Admiral C. F. O. Sankey
History.   *and Surg. Lt. J. B. Sneddon.*
Date

16.8.43.   Primary chancre of coronal sulcus.

16.8.43 to 30.9.43.   N.A.B. 3.75 grams. Bis. 2.2 grams.

24.8.43.   Developed an erythema of arms and chest. Upon one occasion in mid-September some N.A.B. was given into the tissues of the arm; he had a painful arm for a day or so, and a lump remained.

7.10.43.   Given an intravenous arsenical injection, and shortly afterwards the skin on the arm became red, itchy and began to peel. The following day the rash had spread to his face, and on the day of admission his face became swollen and all his skin became red and itchy. No vomiting or nausea. No malaise or diarrhea.

10.10.43.   Admitted to hospital.

Past history.
     Always prone to urticaria. Has suffered from epidermophytosis of the toes in the summer. *Family history.* No allergic diseases. *Condition on admission.* Skin: generalized erythema with commencing desquamation of the face, arms and legs. Some fissures around the toes becoming moist. Gross edema of the face, lips and eye-lids with a purulent conjunctivitis. Mucous membranes normal. Tongue clean and moist. Abdomen: Liver and spleen not enlarged. Heart and lungs: no abnormality discovered.
     White count 11,200.
     Differential count: Polymorphs 49 per cent
                       Lymphs   20 per cent
                       Large monos 6 per cent
                       Eosinophils 25 per cent

A diagnosis of acute dermatitis was made.

Treatment in the first 24 hours was non-specific, fluids being pushed, but no local treatment given.

11.10.43 to 12.10.43.   3.5 grams BAL ointment applied. No erythema or change where the ointment was applied. General skin condition somewhat improved.

13.10.43.   2 grams BAL ointment. Intense subjective irritation after both applications, but no visible change.

14.10.43.   Condition appeared to be deteriorating. Moist areas appearing on the neck, antecubital fossae and feet. Slight oozing on the chest. The condition of the skin continued to deteriorate during the next 3 days, and by 18.10.43 large areas were moist and oozing. At that time a daily saline bath was instituted, and local treatment with ointment applied.

24.10.43.   Developed a temperature of 102.6° and an upper respiratory infection.

26.10.43.   Pain in the chest and an infected sore throat. Started on a course of sulphadiazine. A total amount of 18 grams was given. Skin drying up and general condition improving.

10.11.43.   Skin condition improved, but large areas on the arms, trunk and legs moist and desquamating. General condition rather poor.

12.11.43 to 17.11.43.   1.0 ml. BAL injected intramuscularly.

14.11.43.   Quite definitely improved. Skin less scaly and erythema less. Stated that he noticed a difference even 1 hour after the first injection. No pain in the buttock and no skin eruption.

18.11.43.   Skin had almost regained its natural elasticity, but there was still some secondary infection of the scalp and toes.

20.11.43.   Severe generalized irritation and great difficulty in controlling scratching. However, this settled down with large doses of sedatives. The irritation continued, but got less until 30.11.43, when he developed an urticarial eruption on trunk.

3.12.43.   Skin almost normal.

7.12.43.   Skin normal apart from slight sepsis between the toes.

(Case seen by R. A. P. on 11.10.43. It should be noted that only slight improvement was effected by the inunction on 11.10.43, whereas a dramatic response followed the injection on 12.11.43.)

Case No. 3. Male.   Harrow Road Hospital, London, W.9.
Date   *Major E. J. Mannix, R.A.M.C.*

21.7.42.   Early primary syphilis. W.R. —.

21.7.42 to 2.9.42.   Novostab. 0.45 grams. N.A.B. 1.8 grams. Bis. 2.4 grams. Stabilarsan 2.4 grams.

16.9.42.   Admitted to hospital with arsenical dermatitis. In hospital for 2½ months. Then sent to Convalescent Depot, which he left on 2.1.43.

24.11.42 to 29.1.43. Bis. 3.0 grams.
1.3.43 to 29.5.43. Bis. 3.4 grams.
3.7.43 to 2.10.43. Bis. 4.4 grams.

20.11.43. Acetylarsan 1.5 ml. Bis. 0.2 gram. In good health. Skin well at this time. No other exciting factors.

21.11.43. Itching and spots on thorax.

24.11.43. Re-admitted to hospital. Generalized erythrodermia tending to vesiculation in parts. Treated with mist. alba and calamine cream.

4.12.43. Condition improving.
O.E. face: Dull pink with fine desquation. Minute fissures at angles of mouth and lower lip.
Trunk: Generalized pink, branny rash, stippled, or in places confluent.
Arms: Dry, branny rash.
Legs: Coarser exfoliation, most marked behind knees and on feet.
Hands: Backs thickened and dry. Right palm shows early peeling at base of thumb and index finger, and at wrist. Left hand, similar condition, beginning at base of thumb.
Feet: Soles greasy from ointment, yellowish, unbroken. Toe webs and nails unaffected.
No adenitis. Afebrile. No signs of visceral disease or enlargement. Knee jerks +++. Faint tremor of extended hands. Conjunctivae anaesthetic to coarse pressure. Soft palate scratched without retching. Suggestive anaesthesia to pin-prick.

5.12.43. 2 ml. BAL intramuscularly.

6.12.43. 1 ml. BAL intramuscularly.

14.12.43. No evidence of any skin lesion.

(Seen by W. J. O'D., R. A. P., R. H. S. T., and D. I. W. on 4.12.43, and by W. J., O'D. and D. I. W. on 14.12.43.)

Case No. 4. Male.   Harrow Road Hospital,
                             London, W.9.
Date   *Major E. J. Mannix, R.A.M.C.*

12.10.43. Early primary syphilis. W.R. ++.

14.10.43 to 13.11.43. N.A.B. 3.45 grams. Bis. 1.2 grams.

20.11.43. Mild icterus and rash. Malaise, nausea, heartburn and vomiting (once). No diarrhea or joint pains. Admitted to hospital.

2.12.43. Developed generalized maculo-papular eruption. Complained of nocturnal skin irritation. Seen by Command Dermatologist and diagnosed as arsenical dermatitis.
Condition on admission: Yellow sclerae. Faintly yellow trunk and limbs. A generalized rather coarse eruption, mostly on hips, characterized by irregularly shaped pink splashes, dry and rough, finely papular or small, flat, irregular plaques, with a whitish surface; inside of right arm abraded and red, with sweat dripping from axilla, bluish-pink splashes on shins. Webs of fingers, wrists and penis clear.

8.12.43 to 11.12.43. One ampoule BAL intramuscularly b.d. for 3 days. Rash had been subsiding with some desquamation before treatment started.

9.12.43. Areas of thighs originally clear of rash showed new eruption similar to rest of body.

10.12.43. No irritation. Rash on thighs subsiding.

11.12.43. Rash improving, desquamation well marked.

14.12.43. Rash disappeared. Residual pigmentation present.

28.12.43. Discharged from hospital. No recurrence of eruption, though residual pigmentation persists. The jaundice followed the usual course, and was responsible for the long stay in hospital. There was no general reaction to the injections, and local reaction was limited to slight heat, tenderness and stiffness of the buttock, appearing in 6 hours and disappearing within 24.

31.12.43. Skin smooth, soft and clear, with scattered brown guttate pigmentation of the trunk. Fit for duty.

Serum bilirubin estimations were carried out at intervals to determine whether BAL therapy was exerting any obvious effect on the jaundice. The results, which are given below, do not reveal any striking effects.

Date	*Mgm. bilirubin per 100 ml. serum*
6.12.43	3.9 } before BAL
7.12.43	4.5 }
8.12.43	3.5 } BAL injected
10.12.43	2.5 }
13.12.43	2.1 }
16.12.43	1.2 } after BAL
19.12.43	1.2 }

(Seen by W. J. O'D., R. A. P., R. H. S. T., and D. I. W. on 4.12.43 and by W. J. O'D. and D. I. W. on 31.12.43.)

Case No. 5. Female, aged 33.   Royal Free Hospital,
                                   London, W.C.1.
                                   Dr. M. M. Shaw.

Past history. Diphtheria at age of 10.
Syphilis first diagnosed September, 1942, when in hospital suffering from gummatous ulcers of left thigh and calf. Wasserman 1942, strongly positive. Received 3 injections of arsenic (neo-arsphenamine, 0.3 gram, 0.3 gram, and 0.45 gram) together with bisocyl. 21 to 28.10.42. 0.9 gram neo-arsphenamine, and 2 injections bismuth. 4.11.43. Re-admitted to hospital with 'severe generalized arsenical dermatitis affecting whole body from scalp to toes.' 20.1.43. 'Good recovery and discharged.'

Date

7.6.43. Admitted to surgical ward with (*1*) fracture of right femur, (*2*) compound fracture of left tibia and fibula and (*3*) laceration of calf and right forearm, having fallen from third floor window. While in hospital no active anti-syphilitic treatment, but advised to attend V.D. clinic later.

17.12.43. Attended clinic and received N.A.B. 0.15 gram with 0.45 thiostab.; adexolin ordered.

22.12.43. Returned with second attack of dermatitis. Very distressed and ill. Temp. 96°, P. 132. Admitted to ward and given thiostab. 0.9 gram in 20 ml. glucose. Stated skin irritation began few hours after arsenic injection. General erythema, face flushed and swollen, slight peeling on face and back of neck, eyes puffy, legs edematous and flushed, with early desquamation.

24.12.43. More comfortable but more desquamation, soreness under axillae and breasts. Given thiostab. 0.9 gram in 20 ml. glucose.

26.12.43. Condition worse. General exfoliation.

26 to 28.12.43. BAL injections, 2 ml. twice daily.

29.12.43. Complained of pain at site of injection (gluteal muscle). General exfoliation, but face improved.

30.12.43. Much improved. Face nearly clear. Hands peeling and sore. Back very sore.

5.1.44. Abscess R. gluteal region. Skin clear except for soles of feet.

14.1.44. Skin now completely clear.
Abscess of L. ·gluteal region. Healed well with simple treatment.
No relapse of skin condition noted at any stage.

Note 1: Dr. Shaw has stated (19.1.44) that BAL cleared up the clinical condition in this case of arsenical dermatitis with great rapidity.

Note 2: Note the small amount of N.A.B. causing damage. (Seen by R.H.S.T. on 20.12.43.)

Case No. 6. Male.   Hope ¡Hospital, Pendleton, Salford.

Date   *Dr. A. Gill.*

18.11.43 to 13.12.43. Stabilarsan 5.25 grams. Bismuth 1.2 grams.

16.12.43 to 22.12.43. Thiostab. 1.2 grams daily. Generalized exfoliative dermatitis.

26.12.43 to 28.12.43. BAL injected.

1.1.44. Exfoliation almost complete on trunk and arms. Patient began to feel a little better. Apyrexial. Skin dry. No secondary sepsis.

4.1.44. Mild rigors.

5.1.44. Died suddenly.

6.1.44. Autopsy showed (*a*) Terminal bronchopneumonia. (*b*) Arsenical dermatitis. No evidence of liver cirrhosis.

Case No. 7. Male.   Military Hospital, Preston.

Date   *Major Laird, R.A.M.C.*

15.1.43. Chancroid. Dk. ground neg. (3 times). Eusol-sulphanilamide powder 3/52.

7.10.43. Maculo-papular rash. Small penile sore at fresh site. Generalized adenopathy. Dk. ground ÷.

15.10.43. Kahn ÷.

18.10.43. Kahn ÷÷.

8.10.43 to 7.12.43. Neo-arsphenamine 6.0 grams. Bis. 2.4 grams.

23.12.43. Admitted to hospital with exfoliative dermatitis. All areas including scalp involved. Exfoliation moderately severe. Conjunctivitis and blepharitis present.

26.12.43. Temp. 99°. General condition satisfactory. No change in skin. Pocket of pus superficial to left upper eyelid.

30.12.43. Abscess outer canthus right eye incised.

2.1.44. Skin improved. Abscess just below left eyebrow. Multiple folliculitis. Treated: Calamine and oil. 2 per cent Argyrol to eyes.

On examination: Movement restricted by soreness. Slight cough. Tongue. Mind clear, slow. *Head:* Pink. General, fine branny desquamation; styes, right and left. Small domes of pus on right and left upper lids. Small dry pustules on face.

Upper limbs: Dry, shaggy with peeling. Deep, thick, dry peeling of palms and fingers. Slight folliculitis. Dry and pink round elbows.

Back: Dry; pink; very fine universal peeling. Erupting boils at root of neck and above right shoulder. Sparsely scattered follicular scabs between scapulae.

Front: Harsh and dry. Multiple folliculitis. Boils on mid thorax, groins, pubis and perineum.

Feet: Extensive deep, dry peeling, spreading to toes.

Legs: Coarse, dry peeling. Small boils in both popliteal fossae. Long operation scar astride right thigh (from accident to right femur, age 8). No urethritis. Teeth fairly good. No icterus.

Diagnosis: Severe arsenical dermatitis, complicated by skin sepsis.

7.1.44. Skin still improving.

9 to 11.1.44. BAL, 2 ml. b.d. Skin improving quickly.

14.1.44. Skin very greatly improved. Multiple abscesses increasing in number.

18.1.44. Skin clear of all scaling and erythema, except for scalp and feet. Residual sepsis persisted for 7 weeks.

(Seen by W. J. O'D. and D. I. W. on 3.1.44.)

Case No. 8. Female.   St. Mary's Hospital, Portsmouth.

Dr. A. Murray Stuart.

Arsenical dermatitis.

No previous history of skin affections or sensitivities. Occupation: Housewife.

Date

22.10.43. Attended V.D. clinic with history of labial sore 10 months previously. Mucous patches on lower lip.

22.10.43. Wasserman strongly positive.

29.10.43. Wasserman strongly positive.

CASE No. 8
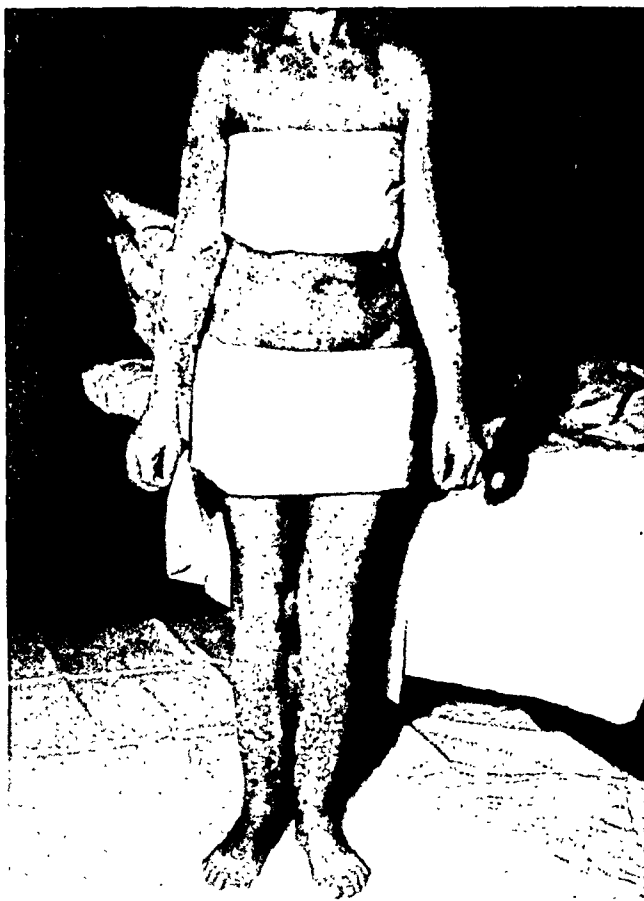
15th January 1944.   15th January 1944.

Taken 11 days before first injection.

5.11.43 to 22.12.43.   Novostab. 4.5 grams.   Chlorostab. 1.6 grams.

31.12.43.   Dermatitis on chest, axillae and groin; treated with calcium thiosulphate.

7.1.44.   Dermatitis very extensive; again treated with calcium thiosulphate and borocalamine lotion.

13.1.44.   Admitted to hospital with generalized exfoliative dermatitis affecting the whole body, except the soles of the feet. Oozing on scalp, inner sides of forearms, inner sides of thighs and legs. Exfoliation of the rest of the skin. Treated with sodium thiosulphate, 10 ml. daily, and 1 ml. Hepatex weekly.

22.1.44.   All treatment discontinued except calamine lotion externally.

26.1.44 to 28.1.44.   BAL injected; 2 ml. daily.

1.2.44.   No improvement yet noted in the condition of the skin, which is still moist in some areas and exfoliating in others. Itching a little less. Temperature, which had risen several times to as high as 101.6°, has subsided to a maximum of 99.8°.

2.2.44 to 10.2.44.   *Another 9 injections of BAL given, 2 ml. daily.*

11.2.44.   Patient now improved very considerably. Says she feels quite well. Temperature remains normal. All the skin areas are dry. Still some desquamation on the nape of the neck, abdomen and backs of forearms. Abdomen markedly pigmented.

14.2.44.   Improvement maintained until 14.2.44, when she began to develop axillary and inguinal adenitis with some superficial inflammation of the left breast. Areas of the scalp and forearms have again become slightly moist. Diagnosed as a secondary staphylococcal infection. Sulphathiozole, 4 grams daily, given for 5 days. Gentian violet applied.

2.3.44.   Skin clear except for lichenification of backs of hands and left shoulder.

11.3.44.   Discharged. Photographs appended.

Case No. 9.   Male.   Harrow Road Hospital, London, W.9.
Date   *Major E. J. Mannix, R.A.M.C.*

2.2.44.   Admitted to hospital, suffering from arsenical dermatitis.

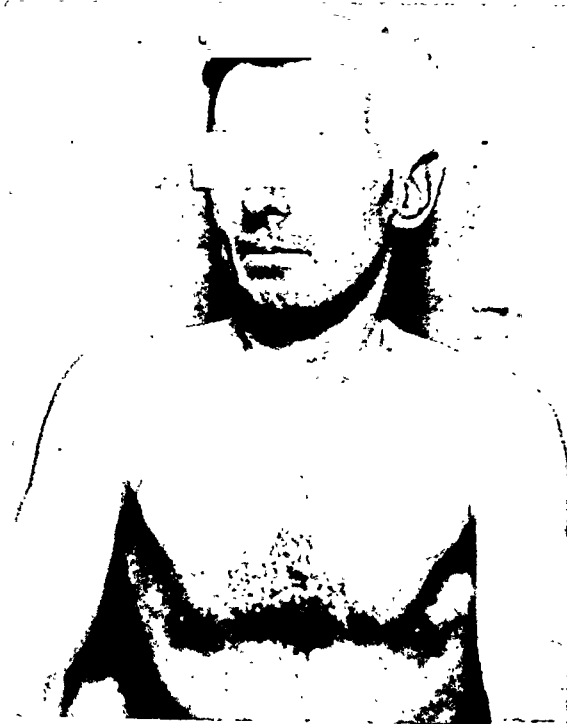5 Feb. 1944.   5 Feb. 1944.

Taken before treatment, on day of first injection of OX.217.

16 Feb. 1944.   16 Feb. 1944.

Taken 11 days after first injection of OX.217.

History: Syphilis 18.9.43. Previous diseases:
Nil. Family history of allergy: Nil.
Occupation: Acetylene welder.
Treatment: With N.A.B., 1st course 22.10.43
to 23.12.43. Total arsenic 4.05 grams. Kahn
weak +.
Second course stabilarsan 21.1.44 to 0.45. 25.1.44
to 0.6. 1.2.44. Arsenical dermatitis.
On admission: Suffered from headache, general
malaise, moderate pruritus. Temp. 100°.
Tongue slightly coated. Conjunctivae normal.
Skin: With the exception of the palms and
soles, which were clear, and the chest on which
there was a general erythema, there was a
generalized dusky erythrodermia of whole body
with fine desquamation tending to be coarser
on the face.
Patient treated with pot. permang. baths, cala-
mine cream and mist. alba.
5.2.44 to 7.2.44. 2 ml. BAL injected daily.
After 3rd injection skin showed marked im-
provement, erythrodermia subsiding generally.
Palms and soles began to peel. Complained of
headache and pain behind eyes.
10.2.44. Skin condition showed signs of relapse, and
daily urine output dropped to 850 ml.
10.2.44 to 12.2.44. BAL 2 ml. daily. With exception of

slight erythema of chest and desquamation on
palms and soles the skin appeared normal.
Urine output, however, remained subnormal.
Large quantities of fluid ordered, and output
rose rapidly, on one occasion reaching 2900 ml.
in 24 hours.
15.2.44. W.R. neg.
On the 19th day after commencement of injec-
tions patient complained of nausea and weakness,
urine output again dropped to 800 ml., and
showed traces of bile and albumen. On 22nd
day developed generalized jaundice.
On 28th day (4.3.44) following commencement
of treatment, a rash appeared on the body, most
marked on the thighs, lower abdomen and back.
A further course of three 2 ml. injections of
BAL was given.
Following the second injection the rash com-
pletely disappeared.
Photographs appended.

Case No. 10. Female, aged 23. Royal Infirmary,
Derby.
Date *Dr. H. R. M. Richards.*
30.12.43. Admitted to hospital with extensive secondary
syphilitic ulceration of the vulva. Severe head-
aches. 8 to 9 months pregnant. (Next day,

CASE No. 10

14th Feb. 1944.
Taken on day of first injection of OX.217.

28th Feb. 1944.
Taken 14 days after first injection of OX.217.
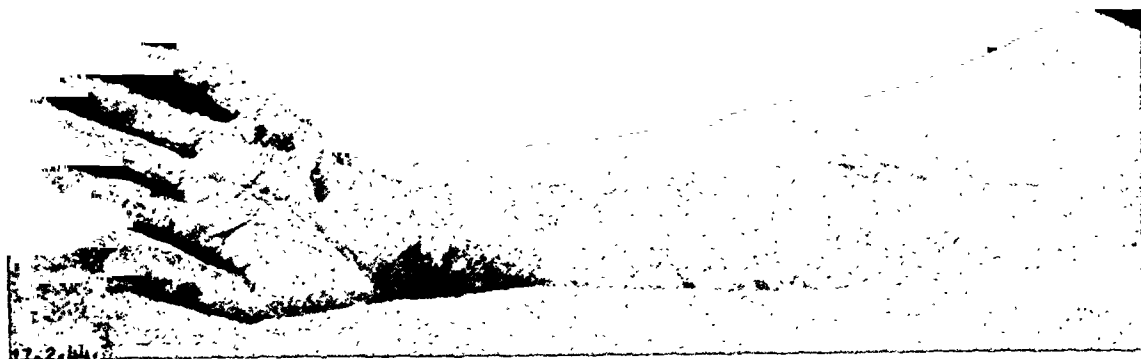
17th Feb. 1944. 3 days after first injection of OX.217.

21st Feb. 1944. 7 days after first injection of OX.217.

delivered of marasmic baby which lived 4 hours.) Treated with N.A.B. 0.3 gram. Bismuth 0.2 gram.

5.1.44. N.A.B. 0.45 gram.

13/20/27.1.44. N.A.B. 1.35 grams. Bismuth 0.6 gram.

3.2.44. Bismuth 0.2 gram. Irritation and erythema of forearms.

9.2.44. More acute and more generalized pruritus. Erythema spread to chest and thighs with some edema of fingers.

10.2.44. Edema spread to forearms and desquamation began, skin of forearms and thighs becoming livid. Felt cold. Irritation more intense, malaise, loss of appetite, some nausea.

12.2.44. Rash more extensive and severe. Skin livid, leathery and desquamating. Increased swelling of fingers and limbs, pruritus and malaise increased. Condition rapidly deteriorating.

14.2.44. Edema spread to face. Dermatitis generally more intense, desquamation increasing, serous discharge from chin, forearms and hands.

14.2.44 to 16.2.44. BAL injections.

15.2.44. Bullae forming on hands and feet, copious discharge from chin, upper and lower limbs and,

to lesser extent, from trunk. Desquamation spreading on face, slight conjunctivitis. Tongue clear.

17.2.44. Improvement in skin condition and appetite. Oozing reduced, and edema of face and arms subsiding.

18.2.44. All lesions dry except on knees and feet. Skin looks healthier despite generalized desquamation and exfoliation of hands and feet. Secondary infection of forearms. Temp. 102.6°. Swab from skin showed staphylococci, streptococci and B. coli.

19.2.44. Edema much reduced, but painful fissures on forearms. Temp. 103°. Slight oozing still on chin, feet and knees. Ungt. thiazamide applied to skin.

21.2.44. Marked improvement. No edema. No sepsis. Skin lighter in colour and desquamation much less marked. Temp. 100°. Feels much better and warmer. Improvement maintained.

28.2.44. Skin dry and returning to normal colour. Scattered scaling, particularly in scalp. Temp. normal, general condition and appetite much improved. Photographs appended.

Case No. 11. Female, aged 30.

Queen Alexandra' Military
Hospital,
Shenley.
Major M. Bolton, R.A.M.C.

No previous history of any skin affection or sensitivity.

Date

7.1.44.   Secondary syphilis manifested by vulval sores, laryngitis, mucous patches. No secondary rash. (No concomitant gonorrhea. No sulphonamides given.) Dk. ground ++. W.R. ++.
The skin of the face was dry and scaly on admission; she declared this was its usual condition.

8.1.44 to 2.2.44. N.A.B. 2.7 grams. Bismostab. 8 grams. No reactions. Complained that about 6 hours after each injection (except first) she felt feverish. Stated she had rash on both anticubital fossae from 26th Jan. (not observed by M.O.).

3.2.44.   Vesicular eruption of arms and shoulders with general erythema and suffusion of conjunctivae. Temp. 99.2°. Gradual increase in extent of vesiculation with maintained low grade fever. Treatment: Zinc cream twice daily and sodium thiosulphate by mouth daily. Pot. permang. baths.

16.2.44. *Condition before treatment:* Patient just out of pot. permang. bath. Plump, well-nurtured, depressed, lachrymose.
Face and scalp: No abnormality.
Conjunctivae: Clear, no suffusion, no icterus.
Neck: General erythema with blotchy areas of dusky blue.
Trunk and limbs: Generalized angry erythema, with scattered patches dusky blue. Weeping extensive over trunk and limbs. Exfoliation beginning over shoulders, inner side of arms and thighs and creases of abdomen. Exudate pouring from these areas. Considerable general edema and subcutaneous edema of feet and ankles.

16 to 19.2.44. BAL injections: 16 and 17 Feb. 2 ml. daily. 18 and 19 Feb. 2 ml. b.d.
Other treatment: pot. permang. baths.

23.2.44. Skin better, drier, with less edema. General condition fair. Complained of pain and great tenderness in both buttocks over site of injection.

25.2.44. Old skin peeling off. General condition poor. Circulation sluggish, with peripheral cyanosis. Edema much less marked.

29.2.44. Skin continues to improve. Obvious abscess R. buttock. Swelling and pain in L. buttock. General improvement.

6.3.44.   Still some dry desquamation, with generalized erythema. Kahn test negative.

9.3.44.   Skin as on 6.3.44. Abscesses incised and pus evacuated.

14.3.44. General condition markedly improved. Skin

generalized dusky red. Scaly patches on forearms and shins, otherwise normal.

28.3.44. Abscesses healed. Skin less erythematous.

25.4.44. Skin normal.

(Seen by D. I. W. on 16.2.44.)

Case No. 12. Male, aged 47. Military Hospital, Preston.

Date *Major Laird, R.A.M.C.*

9.11.43. Primary syphilis. Dk. ground +. W.R. negative.

10.11.43 to 6.1.43. N.A.B. 5.85 grams.

12.11.43. Papular erythematous rash on front of forearms and thighs. Treated for scabies. Gives history of eczema behind ears in 1927 while working with tar boilers.

3.2.44 to 2.42.44. N.A.B. 2.25 grams.

24.2.44. Developed eczema of both arms, and arsenic was stopped.

14.3.44. Admitted with exfoliative arsenical dermatitis. On admission, marked scaling of the scalp and a generalized dermatitis with commencing exfoliation and weeping at the flexures. Temp. 100.4°. Hands and feet not involved.
Treatment: Bed, calamine cream to skin. Hair cut on scalp and ung. sulph. et salicyl. applied.

15.3.44. Skin unchanged. Temp. 98.6°. Complained of sore throat and numbness of hands. Throat slightly infected. Slight swelling of dorsum of hands.

White count, 7400.

Polymorphs	50.3 per cent
L. lymphos	5.6 per cent
S. lymphos	17.6 per cent
Eosins	25.3 per cent
Basos	0 per cent
Trans	0.6 per cent
Monos	0.3 per cent

Phenobarb. gr. 1 nocte.

16.3.44. *On examination:*
Head: Hair short and matted by exudation: retroauricular streptococcal fissures.
Face: Clear. No icterus of conjunctivae.
Arms and trunk: Dusky-red colour. Vesiculation and fine scaling interspersed with areas of complete epidermal loss and weeping. No involvement of palms of hands. Vesiculation of dorsum of hands.
Feet: Normal.
Legs: Gross shedding of skin of thighs and lower abdomen, several flakes up to three inches in diameter. Fine scaling only of legs.
Diagnosis (D. I. W.): Severe exfoliative arsenical dermatitis; a little less severe than Case No. 9 or No. 11.
Prognosis (D. I. W.): 8 to 10 weeks.

17.3.44. Temp. 99°. Exfoliation now generalized, except for hands and feet.

18.3.44. Skin unchanged. BAL 2 ml. b.d.

19.3.44. BAL 2 ml. b.d. Less weeping.

20.3.44. Skin still exfoliating ++. Small abscess in both axillae. Shaved and kaolin applied. BAL 2 ml. b.d.

21.3.44. BAL 2 ml. b.d. Skin still desquamating, but improving slightly.

22.3.44. BAL 2 ml. b.d. Small abscess pubic area.

23.3.44. BAL 2 ml. b.d.

24.3.44. Abscess rt. axilla draining; spirit meth.. and powder applied.

28.3.44. Hands and feet now desquamating. Skin improving steadily. Abscess left axilla dry.

30.3.44. Condition satisfactory. Sepsis in axillae and in pubic region now practically resolved.

31.3.44. Still desquamating.

1.4.44. Skin greatly improved.

4.4.44. Up for half an hour.

12.4.44. Exfoliation complete. Scalp, face and flexures still scaling.

22.4.44. Face and hands still very dry and scaly. Remainder of skin satisfactory.

Both this case and Case No. 7 appeared to be seborrhoeic types.

Both had been treated for scabies. This case has done very well but terminal condition of skin is not so satisfactory as that of Case No. 7. There has been no dramatic improvement of skin that could be attributed to the treatment (seen by D. I. W. on 16.3.44.)

Case No. 13. Female, aged 20. Department of Health, Rotherham.

Date Dr. R. C. Wofinden.

16.12.43. Primary chancre of lt. labium majus of 1 month's duration. Dark ground +. W.R. ++. Kahn ++.

6.1.44 to 10.2.44. Stabilarsan 2.7 grams. Bis. 1.2 grams.

17.2.44. Acetylarsan 3 ml. Bis. 0.2 gram.
On the evening of 17th February patient complained of pain in legs and insomnia. Later, legs began to swell.

18.2.44. Erythematous rash covering whole body, with swelling of face and further swelling of legs. Calamine lotion applied.

24.2.44. Admitted to hospital.
Condition on admission: Afebrile. Pulse 110. R. 20. Marked swelling of face and legs. Commencing desquamation with erythema over whole body including face, arms and legs. Exfoliation over lower thirds of both legs. Thiostab. 0.45 gram i.v., glucose and calamine lotion. No previous skin diseases, no family history of allergy. Patient complained of irritation of

CASE No. 13

Taken before treatment.

Taken 3 days after first injection.

arms. No malaise. Appetite good. No nausea, vomiting or headache. Conjunctivae and tongue normal. No diarrhea. No urine passed first 24 hours after admission. No enlargement of liver or spleen. No albuminuria. No jaundice.

26.2.44. Skin condition unchanged.

29.2.44. Two injections of BAL given. Calamine lotion locally. Fluids ++. Aperients.

1.3.44. Two injections BAL. All edema disappeared.

2.3.44. One injection BAL. Complained of malaise, throat felt slightly sore in afternoon. Temp. 100.5°. Pulse 110. R. 20. O/E throat clear. Chest clear. Sixth dose withheld.

3.3.44. Sixth BAL injection given, general condition improved. Temp. 100°. Pulse 110. Most impressive feature was dryness.

6.3.44. General condition satisfactory. Skin remains dry, and normal skin is appearing beneath exfoliations.

8.3.44. Very depressed. Complained of poor night and being 'wet through' all night. Temp. 97°. Pulse 110. R. 20. Appearances suggestive of relapse.

9.3.44. General condition much improved. Skin dry again and erythema subsiding. Infected crusted patches about ¾ in. in size on each cheek. Starch and boracic poultices applied to cheeks. Pin-point pustules on creases of both palms; treated with fomentations.

11.3.44. Skin condition still improving. BAL not given. Cheeks and palms of hands gradually improved during next few days, and temp. remained normal.

14.3.44. Skin practically recovered.

17.3.44. Oatmeal baths given with rapid removal of remaining desquamation.

23.3.44. Face, body, arms and legs clear. Pain and induration in left buttock.

26.3.44. Fluctuating swelling of upper and outer quadrant of left buttock. Patient afebrile.

28.3.44. Spontaneous discharge of pus through small opening. Incised, evacuated and packed.

31.3.44. Discharged. Still some discharges from buttock.

Note: Dr. R. C. Wofinden has added the following comments (8.7.44).

1. The rapid disappearance of the edema and dryness of the skin following the first 2 injections were quite impressive.

2. The patient had practically recovered within a period of 2 weeks from commencement of treatment. Photographs appended.

Case No. 14. Male.   Harrow Road Hospital, London, W.9. *Major E. J. Mannix, R.A.M.C.*

Diagnosis: Arsenical dermatitis.

Past history of skin conditions: Nil

Family history: Nil.

Present history: 5.1.44 N.A.B. 0.30 gram. 12.1.44 N.A.B. 0.30 gram.

Following the second injection an arsenical reaction was observed. There was a scarlatiniform rash of the hands and feet. Treated with sodium thiosulphate; small doses of N.A.B. were ordered.

Date

19.1.44 to 16.2.44. N.A.B. 2.85 grams.

18.2.44. Developed arsenical dermatitis. First appeared as a blotchy scarlatiniform rash on forearms and hands, less marked on feet.

26.2.44. Slight fissuring and desquamation on dorsum of both feet. Calamine cream applied.

29.2.44. Rash frankly moist and weeping Sod. thiosulphate stopped.

4.3.44. Admitted to hospital.
Condition on admission: Generalized, dusky, red morbilliform rash with yellowish powdery desquamation. The face was swollen and coarsely desquamating. The hands and feet were peeling, and the underlying new skin was erythematous and weeping. The whole picture was complicated by secondary infection. Calamine cream was ordered for the whole body.

6.3.44 to 8.3.44. BAL 2 ml. b.d.

9.3.44. Erythema fading. Much fine desquamation all over body. Hands and feet still weeping.

12.3.44. Generalized weeping worst on lower abdomen and thighs.

12.3.44 to 14.3.44. BAL 2 ml. b.d.

15.3.44. Marked improvement. Hands and feet still peeling and weeping. Erythema on body fading. Desquamation on face clearing up. A number of pustular lesions scattered over the body, particularly on the back and limbs. Ung. resorc. 5 per cent, with sulph. Still a severe degree of pityriasis capitis. Ung. pet. with ol cadi.

20.3.44. Hands and feet no longer weeping.

23.3.44. Allowed up for short time. Complains that feet are tender and painful.

27.3.44. Pustular rash still persisting, but pustules not so numerous.

30.3.44. Ung. pet. with sulph. (scalp). From this until discharge treatment was only for the pustular acneiform lesions on back; all traces of arsenical dermatitis had disappeared.

22.4.44. Discharged.

Case No. 15. Male, aged 21.   Belmont Road Hospital, Liverpool. *Dr. C. McGibbon.*

Past history: No skin trouble. No familial allergy. Generalized secondary macular syphilitic rash with penile chancre 6 weeks ago. Eleven injections of stabilarsan at intervals of twice a week. ? 0.6 gram. given each dose.

Date

28.2.44. Admitted to hospital. Strong, healthy young man with pronounced rash on face, and early

rash on trunk and limbs, 3 to 4 days history. Generalized erythema, face trunk and limbs.

Scalp: Well marked pityriasis capitis.

Face, neck and ears: Dry. Scales tending to accumulate, particularly on forehead. Slight serous discharge behind ears.

Limbs: Extensor surfaces, forearms and thighs show isolated, dry, scaly areas. Early pitting edema of ankles. *W.R.:* Negative. *Conjunctivae:* Clear. *Tongue:* Clean and moist.

Viscera: Nil abnormal.

2.3.44.   Generalized edema, most marked on face and ankles. Purulent conjunctivitis. *Face:* Much more red. Free serous discharge, particularly from forehead. 2 ml. BAL.

3.3.44.   2 ml. BAL b.d. Skin seems drier but still markedly edematous. General condition very good.

4.3.44.   2 ml. BAL b.d. Less edema. Skin much drier. Thick scaling on scalp and face.

5.3.44.   2 ml. BAL b.d. Remittent temperature.

6.3.44.   Much less edema. Increased pyrexia. General condition still very good.

8.3.44.   The gross swelling is subsiding. Skin remains very dry. Cough and sore throat. *Tongue:* Dry. Chest: Nil. *White count:* 10-000. Differential: Nil abnormal.

9.3.44.   Forehead and periorbital region weeping freely. Temp. rising.

10.3.44.   Sore throat worse. Tongue very dry, although taking fluids well and in excellent spirits. Face: Weeping profusely and edema much more pronounced. Can hardly open eyes. Still purulent conjunctivitis, despite frequent irrigations.

Began local treatment for skin: 1 per cent ichthyol and calamine lotion. 2 ml. BAL.

11.3.44.   2 ml. BAL b.d. Increasing erythema of chest.

12.3.44.   2 ml. BAL b.d.

13.3.44.   2 ml. BAL. Throat very sore and dry. Tongue clear and dry.

14.3.44.   Still pyrexial. Throat now giving most trouble. Skin much improved.

15.3.44.   Skin dry and peeling. Some deterioration in general condition. Less erythema. Now has an aphonia. *Throat swab:* Direct smear showed a heavy mixed bacterial flora. No excess of streptococci. No K.L.B. No Vincent's. Culture: Mixed bacterial growth with diphtheroid bacillus predominating.

16.3.44.   Marked decline in general condition. Calciostab. 6 ml. daily (for skin condition).

17.3.44   Very ill. Cannot speak. Throat dry, coated and brown. Commencing white sloughs on posterior pharyngeal wall.

White count: 4000.

     Small monos: 2080 52 per cent
     Large monos: 160 4 per cent
     Polymorphs: 1760 44 per cent
     Eosins

Sodium pentose nucleotide 30 ml. per diem. Morphia gr. ¼ nocte.

18.3.44.   Great extension of white sloughs, covering tongue, palate and pharynx. Facies Hippocratica.

19.3.44.   Patient died.

*Case No. 16. Male, aged 23.*U.S.M.C.

         Lt. Colonel D. M. Pillsbury.

Typical diffuse severe arsphenamine dermatitis, developing after 15th injection of Mapharsen in routine treatment given twice weekly.

Date

24.2.44.   Admitted to hospital with bilateral pneumonia.

6.3.44.   Treatment until this date consisted of 720,000 units of penicillin, 500 ml. whole blood, and 1500 ml. plasma. By 6th March patient had improved greatly in regard to the pneumonic infection. The dermatitis had shown no improvement. 2 ml. BAL given. Diffuse weeping, severe dermatitis.

7.3.44.   2 ml. BAL b.d. Skin paler, almost complete cessation of weeping. Also received 500 ml. plasma for hypoproteinaemia.

8.3.44.   2 ml. BAL b.d. Continued paling of skin. complete cessation of weeping.

9.3.44.   2 ml. BAL b.d. Skin practically normal in appearance. BAL discontinued. The patient later developed some pyoderma which was controlled by oral sulphadiazine therapy and potassium permang. baths.

Case No. 17. Male.   U.S.M.C.
           Lt. Col. D. M. Pillsbury.

Patient received full course of 20 injections of Mapharsen and 8 injections bismuth over a 20 day period for secondary syphilis.

Two days following last injection patient developed generalized erythema of the skin, with considerable itching.

The dermatitis rapidly became more severe, and some exfoliation was noted.

Injections of BAL were given, 4 ampoules of 100 mgm. during the first 24 hours, and 2 ampoules during the second 24 hours.

Marked improvement was noted during the second day of BAL treatment, consisting of subsidence of itching and blanching of the skin.

No further BAL was given and improvement continued. the skin being practically normal on the fifth day after onset of dermatitis.

Case No. 18.   Yeovil V. D. Clinic.
           Dr. D. V. Hague.

Primary syphilis and acute gonorrhea diagnosed on serological findings.

Date

10.1.44.   W.R. + +. Kahn + +.

17.1.44. to 16.3.44.   Stabilarsan 4.8 grams. Bismuth 2.1 grams. Also sulphathiozole.

23.3.44.   Tonsillitis and slight seborrhoeic rash. Bismuth 0.3 gram.

20.4.44.   Arsenical dermatitis. Bismuth. 0.2 gram. Thiostab. 0.45 gram.

27.4.44.   W.R. and Kahn negative. Considerable exudative dermatitis. Thiostab. 0.45 gram.

29.4.44.   Admitted to hospital. Vesicular rash on arms and legs, with edema and slight crusting of exudate. Slight right conjunctivitis. Tongue normal. Slight ulcerative stomatitis. 2 ml. BAL given.

30.4.44 to 3.5.44. BAL 2 ml. b.d. No local or general reaction. .

4.5.44.   Much worse. Generalized rash. Edema of face, both limbs and trunk with crusting exudate. Pyrexia.

5.5.44.   Slight improvement.

11.5.44.   Much worse. Swinging pyrexia, albuminuria, marked edema of face, limbs and trunk with crusted exudate.

14.5.44.   *On examination* (D.I.W.) : Well built brunette whose general condition seems very good.
Hair: Matted with crusted exudate.
Forehead: Closely packed pustules, some broken down and oozing pus.
Face: Covered with dry scales peeling off in flakes the size of a farthing.

Eyes: Purulent conjunctivitis. Slight edema of conjunctivae. Edema of lids. No icterus of conjunctivae.
Neck: Fierce erythema with some scaling of a branny type.
Arms: Bright red; fine scaling; a few areas of active vesicle formation. Fissuring in flexures of wrist, elbow and in axillary folds.
Hands: Normal.
Trunk: Fissuring under breasts and in groins. General erythema; fine scaling; small irregular papules all over trunk.
Legs: Acute vesicular eruption. Shiny, edematous, fiery red skin. No exudation. Edema of ankles and feet.
Feet: Skin normal.
Urine normal; no adventitious sound in chest.

15.5.44 to 16.5.44. BAL 2 ml. b.d.

17.5.44.   Patient very ill, with marked pyrexia and albuminuria. BAL 2 ml.

20.5.44.   Sulphathiazole given.

13.6.44.   Patient has continued to improve since 21.5.44, but with occasional relapses. Skin slowly returning to normal. Dry and scaly all over. Now fairly fit.

(Seen by D. I. W. on 14.5.44.)

CASE No. 19

8th May 1943.	20th May 1943.
Taken immediately before first application of OX.217 ointment.	Taken 12 days after first application of OX.217 ointment.

Case No. 19. Male.   Royal Victoria Hospital,
                          Netley,

Date   *Major D. I. Williams, R.A.M.C.*

28.12.42. Gingivitis. W.R. alleged positive previously.
W.R. repeated 30.12.42: Positive.
Kahn 30.12.42: Negative.
Diagnosed as latent syphilis.

1.1.43 to 27.1.43. Received Mapharside, 0.28 gram.

3.2.43 to 24.3.43. Received N.A.B. 3.6 grams.

24.4.43. Attended hospital, W.R. negative. Kahn nega-
tive.

Acute eczematization of both elbow flexures, with scat-
tered papules on arms and chest (none on legs or face).
Scabies 3 months earlier treated with sulphur and benzyl
benzoate. Present skin condition diagnosed as an early
arsenical dermatitis.

8.5.43. Well marked dermatitis of face, neck, ears,
arms and chest.
Admitted to hospital. Colour photograph taken.
BAL (10 per cent) ointment then applied to
right side of face, neck and chest and to right
elbow flexure; vaseline applied over correspond-
ing areas on the left side.

9.5.43. No apparent change in the condition.

10.5.43. Applications of BAL ointment to right side and
vaseline to left side as on 8.5.43.

11.5.43. General improvement in the condition of the
skin, but no difference detected between right
and left sides.

13.5.43. Still improving.

14.5.43. Right side somewhat better than left.

17.5.43. Some irritation present over the left antecubital
fossa. BAL ointment applied to the affected
part, vaseline to corresponding area on right
arm.

20.5.43. Continued general improvement. Papular ele-
ment markedly receding.

24.5.43. Skin healed. Some xeroderma still present,
probably constitutional.

1.6.43. Skin still healthy. Discharged to light duty.

7.6.43. Re-admitted to hospital, complaining of recur-
rence of skin trouble 3 days after discharge.
On examination, some activity seen over chest,
both antecubital fossae and forearms; condition
only slight with no papular element. On ques-
tioning, patient admitted that arms had been
exposed to sunlight while on duty after dis-
charge.

9.6.43. BAL applied to both sides.

10.6.43. Further application of BAL. Much improved.

14.6.43. Discharged, well.

5.7.43. Seen at hospital, when attending for blood test.
Complained of another slight recurrence 3 days
after discharge (i.e. on 17.6.43) which improved
with calamine lotion and vaseline; on examina-
tion very mild eczematization of both elbow
flexures. Photographs appended.

Case No. 20. Male, aged 39.   Harrow Road Hospital,
                                   London, W.9.

                          Major E. J. Mannix, R.A.M.C.

History: Primary syphilis, March 1943.
Dark ground +. No record of original W.R.

Date

23 to 25.3.43. Mapharside 0.08 gram. Bis. 0.4 gram.

2.4.43 to 18.5.43. N.A.B. 3.0 grams. Bis. 1.4 grams.

18.5.43. Kahn negative.

10.6.43. Bis. 0.2 gram.

17.6.43. N.A.B. 0.6 gram. Bis. 0.2 gram.

24.6.43. Pityriasiform rash on chest, abdomen, thighs
and forearms, observed in patient some 4 days
previously.

24.6.43 to 29.6.43. Bis. 2 grams.

26.6.43. Admitted to hospital.

Treatment and progress.

29.6.43. BAL ointment applied all over affected area.
This caused great stinging and it was washed
off at once; there was a brisk urticarial reac-
tion.

29.6.43 to 12.7.43. Received 28 applications of BAL ap-
plied twice daily over an area of 3 sq. in. of
normal skin on the arms. The skin showed
pigmentation within a few days, which had al-
most subsided within a week, and was gone in
a fortnight.
No other local treatment given.

4.7.43. Developed jaundice. Treated by diet and mist.
alba.

Case No. 21. Male, aged 26.   Harrow Road Hospital,
                                   London, W.9.

                          Major E. J. Mannix, R.A.M.C.

History: Primary syphilis 17.4.43. W.R. ++. Dark
ground +.

Date

17.4.44 to 15.5.43. N.A.B. 3.15 grams. Bis. 1.4 grams.

5.6.43. Admitted with erythema of both forearms,
thighs and calves. During next week feet and
face became very edematous. Eruption on feet
became vesicular and erythematous, and a simi-
lar but milder condition appeared on the face.
Condition of arms meanwhile appeared to sub-
side.

5.6.43 to 28.7.43. Bis. 2.6 grams.

Treatment and progress.

During the first week he was treated with cala-
mine, but when weeping occurred was put on
to pot. permang. baths.

29.6.43. BAL ointment applied to the normal skin of his
chest over an area of 9 inches square. This
caused erythema and some burning. Subse-
quently, therefore, he received inunctions into
normal skin on the arms over areas 3 inches
square. In all, he received 14 applications,
twice daily between 29.6.43 to 5.7.43.

5.7.43. Developed a papular eruption at the site of inunction. His feet were relapsing. Inunctions were stopped.

30.7.43. He is now well.
Inunction had no beneficial effect.

Case No. 22. Male.
Surgeon Comm. E. G. Thomas, R.N.V.R.

Date

16.5.43 to 19.7.43. Treatment for sero-positive syphilis. N.A.B. 4.05 grams. Bicreol. 12 ml.

27.7.43. Admitted to hospital on account of arsenical dermatitis. Condition was generalized and severe; the face was edematous, the trunk and limbs were a bright red colour, and on the skin were numerous vesicles and pustules.

31.7.43 to 2.8.43. 5 per cent BAL ointment rubbed in daily, over different areas of only mildly affected skin on the trunk.

Little change noticed until 7.8.43.

10.8.43. General improvement remarkable. Edema ha disappeared, the skin of the trunk is rapidl approaching normal, and the limbs are dry an show some scaling.

26.9.43. Dermatitis recurred on forearms, and rapidl became generalized, exfoliating as in the pre vious attack.

2.10.43 to 4.10.43. Treated with BAL ointment. Condition improved gradually.

(Seen by R. H. S. T. on 31.7.43.)
Photographs appended.

Case No. 23. Male. Harrow Road Hospital, London, W.9.
Major E. J. Mannix, R.A.M.C

History.

Date

6.7.43. Primary syphilis. W.R. ++.

CASE No. 22

2.8.43.   2.8.43.

Two days after the first inunction of OX.217 ointment.

12.8.43.   12.8.43.

Twelve days after the first inunction of OX.217 ointment.

6.7.43 to 25.8.43. 8 injections neoarsphenamine with Bi.

1.9.43.   Erythematous rash on external surface of fore-arm. Treated with calamine cream.

5.9.43.   Admitted to hospital with arsenical dermatitis. There was a papular rash on both forearms and arms, and on both knees, where there was some weeping. Rt. knee worse than left.
Chest, abdomen and buttocks were clear.
Backs of calves and ankles showed a moist dermatitis.

Treatment and progress.

7.9.43 to 13.9.43. Twelve inunctions of BAL ointment. The ointment was rubbed thoroughly into an area of healthy skin (about 2 in. in diameter) in the shoulder area, twice daily. Patient complained of some stinging a few minutes after each inunction. Skin of knees and ankles breaking down. From 10.9.43 to 13.9.43 these areas were covered with saline dressings.

13.9.43. Patient obviously worse, with weeping areas on right knee and dorsum of left ankle and scattered patches on arms and legs.
BAL treatment was stopped, and he was put on pot. permang. baths and lin. cal. to the quies-

cent patches. He showed improvement within 24 hours.

20.9.43. Skin peeling from palms of hands and soles of feet, but apart from slight erythema of the knees there were no signs of activity.

2.10.43. Discharged. No beneficial effect of the BAL inunctions observed.

Case No. 24. Female, aged 23.   County Hospital, York.

Date   *Dr. S. G. Platts.*

15.10.43. Peeling of skin following sixth injection of 0.45 gram stabilarsan. The dermatitis spread rapidly, involving most of the body, and being especially bad on face and legs.

22.10.43. Admitted to hospital.

29.10.43 to 8.11.43. BAL ointment applied over small areas of healthy skin on arms, feet, hands, breasts and loins.

31.10.43. Improvement in generalized rash. Patient can now open the eyes.

10.11.43. Temp. 103°. Some secondary infection on cheeks and in the bends of the elbows. Ung. sulphathiazole applied to the affected areas. Desquamation has now ceased.

CASE No. 24

2.11.43.

Four days after the first inunction of OX.217 ointment.

25.11.43.

Twenty-seven days after the first inunction of OX.217 ointment.

15.11.43. Temperature settled.
26.11.43. Discharged from hospital.

Patient's mother has also had an attack of arsenical dermatitis. It was less severe than the present case, but took at least 3 months to clear, though there was no delaying secondary infection.

Photographs appended.

Case No. 25. Male, aged 44.   Belmont Road Hospital
Liverpool.
Dr. C. McGibbon.

Papular syphilide. W.R. negative.

Date
7.10.43.   Stabilarsan 0.45 gram and bismuth.

7.10.43 to 5.11.43. Marpharside 0.06 gram given 3 times each week, until 14 injections had been given.

5.11.43. Exfoliative dermatitis. Treated as outpatient for 9 days. Ca gluconate given i.v. daily. Admitted to hospital.

Condition of admission: Generalized erythema, with fine, dry, branny scales over the whole body, more marked on abdomen, face and scalp. Treated with BAL ointment, this being applied to the abdomen and upper arms on 3 consecutive days. Within 1 to 2 days of each application there was a noticeable clearing of the scales, and to a lesser extent of the erythema, for a radius of about 3 to 4 in. from the site of application.

Seven days after the first inunction, as the face was still scaly, the ointment was applied to the cheek and forehead on 1 side of the face only, once daily for 3 days; this time there was a marked contrast between the treated and untreated halves of the face, the former being almost free from scales, while the latter was still thickly scaly; the abdomen showed a slight return of the scaling, but not nearly so marked as on admission.

Complete recovery 21 days after the first inunction; no other local treatment given. General condition excellent throughout; no serous discharge from the skin at any stage.

Case No. 26. Male, aged 46. Belmont Road Hospital, Liverpool.
Date Dr. C. McGibbon.

19.11.43. Diagnosed as secondary syphilis.
Generalized macular roseolar rash present for 7 days.
W.R. strongly positive.
No history of previous skin trouble.
N.A.B. 0.45 gram, alternating with 0.3 gram twice daily.
Two days after the 11th injection (*i.e.* after 4.05 grams of N.A.B.) he developed a generalized erythema. This was not diffuse, but appeared to follow the pattern of the subcutaneous blood vessels. The rash faded on pressure. No papules, vesicles or scaling.
BAL ointment applied to abdomen once daily for 4 days. By the end of the treatment the erythema was only slight.
Six days later, *i.e.* 10 days after the commencement of treatment, the skin was normal.

Case No. 27. Male. Royal Infirmary, Liverpool, 3.
Dr. Ross.

Sero-positive primary syphilis.
Date
25.9.42 to 2.11.42. Mapharside 0.6 gram. Bismuth 1.2 grams.

4.11.42. Blood W.R. negative.
16.2.43. Blood W.R. negative.
22.2.43 to 1.3.43. Mapharside 0.14 gram. Bismuth 0.3 gram.
5.3.43. Became jaundiced. Icteric index 30.
13.8.43. Icteric index 7.
18.8.43 to 2.11.43. Mapharside 0.36 gram. Bismuth 1.8 grams.
15.11.43. Developed a maculo-erythematous arsenical dermatitis involving trunk and limbs.
Treatment with ung. sulph. and daily injections of calcium thio-sulphate and liver extract resulted in no improvement.
24.11.43 to 25.11.43. BAL ointment applied twice daily.
1.12.43. Dermatitis has all but gone, though irritation is still bad at night. Calamine lotion prescribed.

Case No. 28. Male. Health Department, Bridgewater.
Dr. G. H. Pringle.

History. Primary syphilis in young adult male.
Date
15.9.43 to 3.11.43. Stabilarsan 3.6 grams. Bis. 1.6 grams.
10.11.43. Dermatitis. Injection thiostab. given.
Dermatitis gradually became worse. Sodium thiosulphate injections had no effect.
1.12.43. Severe generalized exfoliative dermatitis. Face swollen, with blistering on the face and in the flexures. Hematuria present. The skin was pigmented and the patient complained of dryness of the mouth. No other constitutional symptoms.
4.12.43. Admitted to hospital.
4.12.43 to 7.12.43. 2 ml. BAL b.d. intramuscularly into the buttocks.
14.12.43 Two abscesses developed at injection sites.
23.12.43. Abscesses opened and drained under general anesthetic.
18.1.44. Marked steady improvement in the patient's general and local condition since 23.12.43.
The only local treatment was calamine lotion and olive oil, with the exception of 1 bath in 1 in 8000 pot. permang.
The condition is now almost clear.

Case No. 29. Male, aged 51. Health Department, Nottingham.
Dr. R. Marinkovitch.

Plate-layer by occupation. Has never suffered from eczema or asthma. No history of asthma in the family.
Date
5.11.43. Attended for treatment of gummatous ulceration of R. elbow. W.R. ++.
15.11.43 to 21.1.44. Neoarsphenamine 4.35 grams. Bismuth oxychloride 2 grams.
W.R. strongly positive after completion of this course of therapy.

12.2.44 to 25.3.44. N.A.B. 3.15 grams. Chlorostab. 1.4 grams.

1.4.44. Developed erythematous, itchy eruption on the legs (below the knees), and on forearms and face.

Calamine lotion applied.

8.4.44. Generalized erythema. Oozing and crusting of the skin on the neck, antecubital fossae, forearms, axillae, scrotum, lumbar region and legs below the knees.

15.4.44. Ears swollen, red and oozing. Scalp scaly. Edema and erythema of the face. Neck, axillae, lumbar region and scrotum exfoliating and crusting; neck very irritable. Forearms and legs angry-looking. Secondary infection in the lumbar region. Mucous membranes of the mouth and eyes grossly affected. Viscera, nil abnormal. Urine, no albumin.

19.4.44. BAL injections commenced. 2 ml. b.d. for 4 days.

20.4.44. Patient complained of generalized irritation. Mild pyrexia, 100.8°. No change in the skin condition.

21.4.44. Marked change noticed. Erythema, swelling and oozing disappeared. Desquamation and exfoliation increased all over body.

22.4.44. Patient comfortable. Skin desquamating and exfoliating in large flakes. Edema of the face and legs gone.

29.4.44. Relapsed. Erythema and intense irritation on the legs, back and neck. Local treatment commenced.

5.5.44. Erythema gone. Lichenification and exfoliation on the posterior aspect of neck, and on the antecubital fossae and legs. Exfoliating on forearms, legs and lumbar region.

TABLE VI
Data on arsenical excretion

Case	Before injection of BAL		During injection of BAL		After injection of BAL	
		mgm. As per diem		mgm. As per diem		mgm. As per diem
1	Oct. 31 to Nov. 1	0.351	Nov. 3 to 4	0.196	Nov. 6 to 7	0.221
	Nov. 1 to 2	0.249	4 to 5	0.139	7 to 8	0.092
	2 to 3	0.150	5 to 6	0.221		
			8 to 9	0.131		
			9 to 10	0.200		
2	Nov. 10 to 11	0.168	Nov. 12 to 13	0.193		
	11 to 12	0.143	13 to 14	0.180		
			14 to 15	0.208		
4	Dec. 6	0.538	Dec. 8	0.530	Dec. 11	0.583
	7	0.787	9	0.775	12	0.456
			10	0.708		
5	Dec. 24	0.485	Dec. 27	0.297	Dec. 30	0.202
	25	0.275	28	0.166	31	0.160
	26	0.236	29	0.186	Jan. 1	0.245
					2	0.119
6	Dec. 26 to 27	0.362	Dec. 28 to 29	0.347		
	27 to 28	0.298	29 to 30	0.379		
			30 to 31	0.246		
7	Jan. 4 to 5	0.493	Jan. 8 to 9	0.437	Jan. 11 to 12	0.286
	5 to 6	0.529	9 to 10	0.291	12 to 13	0.251
	6 to 7	0.129	10 to 11	0.435	13 to 14	0.232
	7 to 8	0.350				
8	Jan. 22	0.607	Jan. 25	0.396	Jan. 29	0.233
	23	0.339	26		30	0.274
	24		27	0.354		
			28		Feb. 10	0.094
9	Feb. 3 to 4	1.190	Feb. 5 to 6	0.848	Feb. 8 to 9	0.650
	4 to 5	1.470	6 to 7	0.750	9 to 10	0.527
			7 to 8	0.945	10 to 11	0.576
					11 to 12	0.340
			Feb. 12 to 13	0.450	Feb. 15 to 16	0.520
			13 to 14	0.390	16 to 17	0.305
			14 to 15	0.520	17 to 18	0.555
					18 to 19	0.250

TABLE VI—*Continued*

Case	Before injection of BAL		During injection of BAL		After injection of BAL	
		mgm. As per diem		*mgm. As per diem*		*mgm. As per diem*
10	Feb. 13	0.398	Feb. 14	0.588	Feb. 17	0.777
			15	0.958	18	1.310
			16	1.120	19	0.690
					20	1.030
11	Feb. 14	1.93	Feb. 16	0.78	Feb. 20	0.72
	15	0.59	17	0.87	21	0.82
			18	0.89	22	0.65
			19	0.75	23	0.35
12	Mar. 14 to 15	0.24	Mar. 18 to 19	0.41	Mar. 24 to 25	0.30
	15 to 16	0.45	19 to 20	0.50	25 to 26	0.39
	16 to 17	0.26	20 to 21	0.31		
	17 to 18	0.93	21 to 22	0.38		
			22 to 23	0.37		
			23 to 24	0.33		
13	Feb. 26 to 29	1.050	Feb. 29	0.744	Mar. 3 to 4	0.180
	3 days		Mar. 3		4 to 7	0.437
15	Mar. 4 to 5	0.134	Mar. 6 to 7	1.16	Mar. 9 to 10	0.35
		100 ml.				
	5 to 6	0.076	7 to 8	0.96	10 to 11	0.57
		100 ml.				
			8 to 9	0.67	11 to 12	0.42
			12 to 13	0.16		
			13 to 14	0.32		
16	Feb. 29	2.56	Mar. 2	2.47	Mar. 6	1.17
	Mar. 1	1.90	3	1.97	7	0.97
			4	2.56	8	1.04
			5	0.96		

		As. μg. per gram
Case 2	Before Injection	9.2
	During Injection	8.2
		7.0
Case 7	Before Injection	8.0
Case 8	Before Injection	2.8
Case 10	On day of first Injection	6.3
Case 12	Before Injection	3.5
Case 16	(not known)	9.2

12.5.44. Exfoliation continues.

19.5.44. Desquamation on back, neck, axillae, scrotum, legs and lumbar region. Reaching subacute stage.
Sedatives administered for irritation.

26.5.44. No change.

3.6.44. Irritation and oozing on chest and forearms, lichenification of neck. Soothing creams applied. Sedatives for irritation.

7.6.44. Patient feels comfortable. Skin still irritable. New area of erythema on the back of the chest, with some exfoliation. Slight desquamation.

Case No. 30. Male. U.S.M.C.
Lt. Col. D. M. Pillsbury.

After 6th injection of Mapharsen, developed chills, fever of 101° and a pink, plaque-like type of eruption involving the upper half of the body.

The eruption gradually became generalized.

20.3.44. Initial injections of *BAL* given. After one injection almost immediate mottled blanching was noted, and his temp. dropped 4 degrees to 101°.

Two further injections were given during the next 24 hours, and complete blanching of the skin was noted.

APPENDIX II

EXCRETION OF ARSENIC

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Introduction.

Arsenic analyses upon specimens of urine and of skin exfoliations from several cases of arsenical dermatitis have been done.

Analytical methods.

All estimations were carried out by the method previously described (24), using the large scale procedure with $AsCl_3$ distillation. This was necessary in the case of skin, in spite of the small weights of samples, because of the difficulty in completely digesting this tissue. Analyses were carried out in replicate wherever possible, and especially when unusual results were encountered.

Urine.

It has been shown experimentally that injection of BAL will increase the excretion of arsenic in the urine of an animal poisoned with a trivalent arsenical. It is natural, therefore, to think that this will happen in patients suffering from arsenical poisoning: Eagle (21) has claimed that injection of BAL in oil increases excretion in man. Sample from 24-hour specimens of urine from some of the cases which have been described above, were sent to Edinburgh for analysis. The results are indicated in Table VI. In some cases there is a slight indication of correlation between an injection and a rise in excretion; but this may not be significant, owing to the large fluctuations always present. The general conclusion is that BAL in the doses given had no effect on the urinary elimination of arsenic in these cases.

SKIN EXFOLIATIONS

Specimens of skin exfoliations have been collected in pill boxes and sent to Edinburgh for analysis; the values obtained varied from 3.0 to 9.0 μg. As per gram. Though these estimations do not permit of conclusions as to the progress of the cases, they are put on record as we can find accounts of no other similar estimations, and as they establish the presence of amounts of arsenic in the skin capable of causing damage to the cells.

Though we cannot find records of arsenic estimation in exfoliations, there are accounts in the literature of the arsenic content of hair. Reference to some of these has been recently made by Young and Smith (25), who have themselves analysed hair from cases of arsenical poisoning. The values obtained by them varied from 7.7 μg. to 3.2 μg. per gram hair (dry weight); the higher value was for the proximal portion of scalp hair following a period of fatal illness of only 30 hours. The values are of the same order as those found for the skin exfoliations.[2]

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[2] Thanks is expressed to G. W. Abbot for his valuable technical assistance.

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(M. of S. = U. K. Ministry of Supply.)

CLINICAL USES OF 2,3-DIMERCAPTOPROPANOL (BAL). VII. THE TREATMENT OF ARSENICAL DERMATITIS WITH PREPARATIONS OF BAL

By W. T. LONGCOPE, JOHN A. LUETSCHER, JR., M. M. WINTROBE, AND VAL JÄGER

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The development of 2,3-dimercaptopropanol (British Anti-Lewisite, BAL) by Peters, Stocken, and Thompson (1) has made available a potent antidote for arsenical poisoning. Study of the chemical combination of arsenic with sensitive enzymes and cells indicated that the arsenic could, under certain circumstances, be removed from the cells, and that enzymes could be reactivated by the action of BAL (1, 2). BAL administered to animals exerted both protective and therapeutic effects against local and systemic injury by toxic arsenicals (1, 2). These observations strongly suggested that BAL might be useful in arsenical poisoning in man, and cautious trials were initiated in this hospital at the end of 1942.

METHODS

Patients were admitted to a special ward which was in part arranged for the care of civilians employed at Edgewood Arsenal, under contract between the Johns Hopkins Hospital and the U. S. Employees Compensation Commission, and in cooperation with the Chemical Warfare Service. A detailed clinical study was made of these patients both before and after the administration of BAL. BAL was given by inunction in 1 to 10 per cent ointment (3) and later by intramuscular injection in 5 to 10 per cent solution in peanut oil containing benzyl benzoate (4). Dosage was at first varied and rather small because of the lack of information on the therapeutic and toxic effects of BAL.

Arsenic in the urine was determined by the method of Magnuson and Watson (5) in the laboratory of Dr. H. Eagle. Chemical analyses of the blood were made under the direction of Dr. Mary Buell.

RESULTS

1. Dermatitis due to diphenylamine chlorarsine

A number of workers exposed to the dust of this chemical (also known as Adamsite or DM)

developed intractable dermatitis involving the exposed areas of the face, neck and arms. Most of these patients gave a positive patch test to DM. The itching, burning, erythematous and papular eruption proved resistant to ordinary forms of treatment. They were therefore transferred from Edgewood Arsenal to our special ward for investigation. In 6 of the 7 patients, the dermatitis had persisted for 18 to 50 days before they were admitted to the hospital.

The application of BAL in ointment caused intense burning of the affected areas, but this lasted for less than an hour, after which the patients received great relief from the previous itching and discomfort. Daily applications were made, beginning with as low as 100 mgm., and increasing to 500 mgm. per day in each case. The dermatitis cleared completely in from 2 to 8 days, with an average of a little over 5 days. The patients served as their own controls in these studies, since the eruption had been present for very different lengths of time before treatment, and had failed to respond to other forms of therapy (removal of irritant and application of boric ointment and BAL ointment base) prior to the use of BAL.

In attempting to conduct controls during the course of these observations, BAL in ointment was applied to the lesion on one arm, and the ointment without BAL to the lesion on the opposite arm. Both lesions healed with about equal rapidity. It was known that BAL is absorbed readily through the skin and it therefore occurred to us that the improvement might be due to the systemic action of BAL as well as to its local effect. BAL was therefore applied to unaffected portions of the skin. These inunctions caused no discomfort and therefore comparatively large amounts of ointment could be employed. The results were highly satisfactory, and the dermatitis

cleared under this method of application as rapidly as when the inunction had been made to the eruption itself.

The arsenic excretion in the urine increased during treatment (6), suggesting that arsenic had been released from combination with the cells.

2. Arsenical dermatitis occurring during anti-syphilitic therapy

Fifteen patients referred to the hospital from several clinics because of exfoliative dermatitis produced by anti-syphilitic arsenical drugs have been treated with BAL. Four of these patients received daily inunctions of BAL in ointment (5 to 10 per cent) for periods of 3 to 11 days. The average daily dose of BAL was 500 mgm. The application of this ointment was extremely painful and in one instance so agonizing that morphine was required. The exquisite pain lasted from ½ to 2 hours, but the relief from itching and burning was so marked within 12 to 24 hours that the patients often asked for further treatments. Applications had to be made over different areas each day, otherwise there developed a local pustular dermatitis due, we thought, to BAL itself. In many instances there was a dramatic improvement

FIG. 2. CASE 4, TABLE I

On May 17, 1943, 4 days later than Figure 1, after 1 gram BAL in ointment.

within 48 hours in the arsenical dermatitis (Figures 1 and 2).

This was often most noticeable over the area anointed.

Eleven patients were treated with intramuscular injections of BAL in 5 to 10 per cent solution in peanut oil and benzyl benzoate. The daily dose of BAL varied from 100 to 450 mgm. a day, the average being 300 mgm. Individual injections ranged from 100 to 200 mgm., averaging 150 mgm. One or more courses of treatment lasting 4 to 10 days were given.

Three patients had disagreeable symptoms from 20 minutes to 1 hour after injection of BAL. One patient complained of aching pains in the muscles of the legs, and one patient had burning of the mouth, with nausea and vomiting. These 2 patients were receiving 3.5 and 3.7 mgm. of BAL per kgm. of body weight at 6-hour intervals.[2]

FIG. 1. CASE 4, TABLE I

Showing exfoliating dermatitis of face on May 13, 1943.

[2] While our studies were in progress, the effects of injection of large doses of BAL in peanut oil were tested in human volunteers (4, 10, 11). The results demonstrated that a single dose of more than 3 mgm. of BAL per kgm. of body weight is likely to cause distressing symptoms, but that injections could be repeated at inter-

TABLE I—Treatment with 5 per cent and 10 per cent BAL in ointment

Date of adm.	No.	Sex	Age	Color	Arsenical	Amt.	Doses	Onset of dermatitis after last dose	Duration of dermatitis before adm.	Extent of dermatitis	Duration of treatment	Total BAL	Relapses	No. of courses of BAL	Duration of dermatitis after 1st dose BAL	Complications
						grams		*days*	*days*		*days*	*grams*				
2/25/43	1	F	19	C	Neoarsphenamine	3.0	5	3	11	Generalized patchy, exfoliative. No fever	1st C. 11 / 2nd C. 3 / Total 14	4.0}5.5 / 1.5}	1	2	19 days	None
3/31/43	2	F	22	C	Neoarsphenamine	2.7	5	7	12	Exfoliative, generalized, mod. T. 101 to 102	4	3.0	0	1	12 days	Cutaneous abscess
5/ 4/43	3	F	40	W	Neoarsphenamine 2nd course	0.45	1	1	10	Generalized, exfoliative T. 101	1st C. 5 / 2nd C. 3 / Total 8	2.5}4.0 / 1.5}	1	2	16 to 22 days	Cystitis
5/12/43	4	F	20	C	Diarsenol 2nd course	0.3	1	1 to 8	5	Exfoliative face	4	2.0	0	1	6 days	None

Symptoms disappeared when injections were stopped in one case and the dosage reduced in the other. A third patient had nausea ½ hour after each injection of a small dose of BAL (1.8 mgm. per kgm.). This patient had jaundice as well as dermatitis, and was probably more sensitive to gastrointestinal disturbance (1). One patient developed an abscess of the buttock at the site of an injection, an unexpectedly rare occurrence considering the distressing condition of the skin through which the injection must be made and the frequency of spontaneous abscesses.

The results are presented in Tables I and II. It is impossible in such a small series of cases to evaluate the effects of route of administration or dosage. The results appeared to be beneficial in all cases as judged by the patients' subjective reactions. Itching and burning often diminished within 24 hours, and were greatly improved in 48 hours. At about this time objective improvement in the skin usually became apparent and sometimes progressed to rapid and complete healing of the dermatitis within as few as 6 days. More often some evidences of the dermatitis persisted and complete healing occurred only after several weeks. During this time the patients' complaints were much relieved, and the skin showed only residual changes rather than continuation of inflammation. The dermatitis in 5 patients was prolonged because of mild relapses following cessation of treatment. These relapses usually responded to a second and even third course of BAL. We received a strong impression that treatment should be continued for at least 6 days and preferably longer, since ½ of the patients treated for less than 6 days developed a relapse of the dermatitis, while only 2 of the 9 patients treated 6 days or more showed a recurrence of the dermatitis.

The clinical improvement of these patients was accompanied by a considerable increase in the excretion of arsenic in the urine (6). A similar increase followed a second or third course of BAL during treatment of the relapses.

The observation that relapses of the dermatitis might occur when treatment by BAL was dis-

vals of 3 to 4 hours with a minimal cumulative toxicity, an important point since large amounts of BAL may be given in this way.

TABLE II

Treatment with 5 per cent to 10 per cent BAL intramuscularly

No.	Date of adm.	Sex	Age	Col.	Arsenical	Amt.	Doses	Onset of dermatitis after last dose	Duration of dermatitis before adm.	Extent of dermatitis	Duration of treatment	Total BAL	Relapses	No. of courses of BAL	Duration of dermatitis after 1st dose BAL	Complications
						grams		days	days		days	grams				
1	5/4/43	F	38	C	Neoarsphenamine 2nd course. History of dermatitis	0.3	1	1	8	++++ Generalized exfoliative T. 102 to 105	1st C. 3 2nd C. 3	0.3 0.45 }0.75	1	2	14 days	Infection B. hem. streptococcus and staphylococcus. Sulfadiazine
2	5/12/43	F	25	W	Mapharsen 2nd course. History of dermatitis	0.015	2	Day of last dose	2	++++ Generalized edema, erythema papular T. 102	4	0.85	0	1	9 days	
3	11/25/43	F	19	W	Neoarsphenamine 2nd course	3.6	6	Day of last dose	10	++++ Generalized exfoliative, red T. 99 to 101	1st C. 4 2nd C. 3 3rd C. 3	0.9 0.45 }2.85 1.5	2	3	39+ days	
4	3/10/44	F	38	C	Mapharsen 2nd course	0.30	5	2	8	++ Vesicular, arms and face	6	1.35	0	1	12 days	Jaundice
5	6/19/44	F	25	C	Neoarsphenamine	3	7	3	8	++++ Generalized erythema, papular T. 102 to 105	1st C. 6 2nd C. 6	1.86	1	2	51 days	Jaundice fever, relapsing
6	7/11/44	M	43	C	Mapharsen	1.50	25	3 before last dose	10	++++ Generalized acute, red, exfoliative T. 101	8	1.8	0	1	53 days	Multiple abscesses of skin and lymph nodes
7	10/16/44	F	37	W	Neoarsphenamine	0.9	3	11 ?	11	++ Generalized maculo-papular erythema weeping T. 101 to 103	6	1.8	0	1	17 days	
8	11/14/44	F	19	C	Neoarsphenamine	1.9	4	8	15	++++ Generalized exfoliating edema T. 102 to 105	6	2.35	0	1	80 days	Perineal abscess, Abscesses of skin, multiple; fever 2 months. Penicillin
9	4/24/45	M	36	C	? 2nd course. History of dermatitis	?	3	After 2nd dose	3	++++ Generalized edema, erythema papulo-vesicular T. 99.6 to 101	10	4.3	0	1	13 days marked improvement	Abscess of buttocks
10	5/15/45	F	16	C	Neoarsphenamine	?	6	After 1st dose	21	++++ Generalized vesicular crusting, weeping T. 100.8	7	2.85	0	1	8 days	
11	4/2/45	F	25	C	Neoarsphenamine	2.4	4	2	35	++++ Generalized T. 100.2	8	1.8	0	1	16 days	

continued, but could be controlled by reinstituting treatment, and that the excretion of arsenic in the urine increased during each course of treatment by BAL, but decreased rapidly each time that the administration of BAL was omitted. suggested very strongly that there was a relationship between the 2 sets of conditions. Such a correlation might be interpreted as an indication that the intramuscular injections of BAL resulted in a liberation of arsenic from the tissues of the body, and that this release of arsenic was followed by clinical improvement.

DISCUSSION

We have been very favorably impressed with the response to the administration of BAL in exfoliative dermatitis. The patients described a considerable relief of symptoms. The objective improvement was often equally impressive, but complete healing was frequently delayed for several weeks. It may be possible to eliminate the mild

relapses which prolonged the course of ⅓ of our patients, by continuing the treatment for as long as a week or more after the initial rapid improvement occurs.

In addition to the relief of the patient's symptoms and the objective improvement in the skin after the use of BAL, we had the impression that the patient's illness was shortened. Because of the variability of the disease, it is difficult to obtain any accurate information on the duration of arsenical exfoliative dermatitis. It is said to last "seldom less than 8 weeks and often as long as 12 or more" (7).

During the past 20 years, 49 patients have been admitted to the Johns Hopkins Hospital for arsenical exfoliative dermatitis, and have been followed throughout the course of the disease in the hospital and its out-patient department. The precipitating agents were neoarsphenamine (23 cases), arsphenamine (10 cases), mapharsen (3 cases), and in several cases the less commonly used
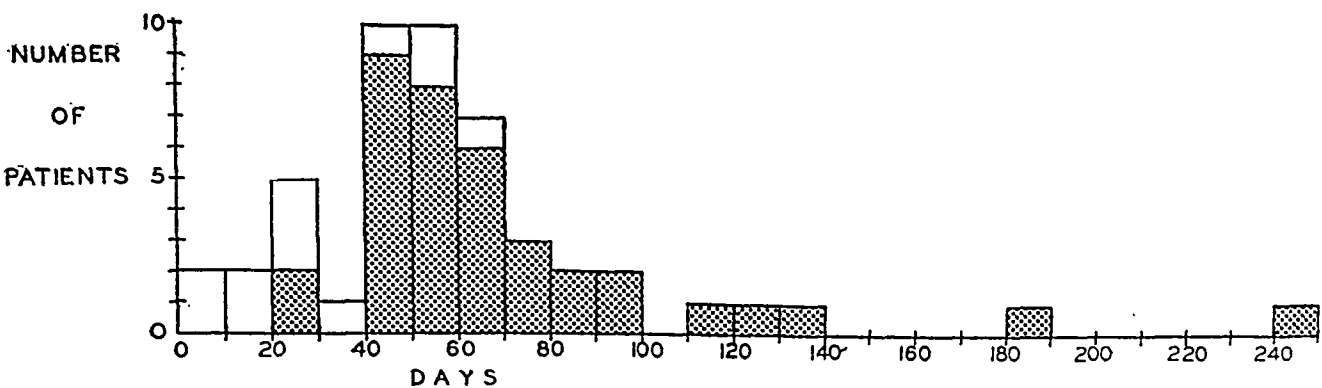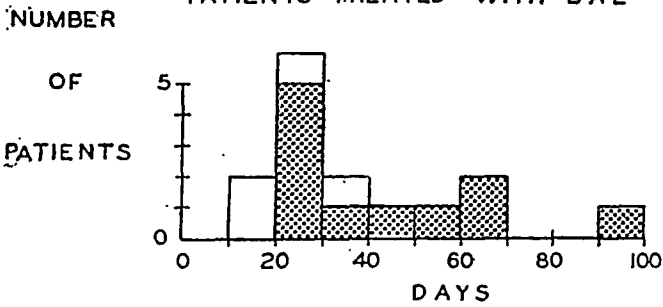

FIG. 3. COMPARISON OF THE TOTAL DURATION OF EXFOLIATIVE DERMATITIS IN 49 ATTACKS IN 46 PATIENTS NOT TREATED WITH BAL, WITH THE TOTAL DURATION IN 15 COMPARABLE PATIENTS TREATED WITH BAL

Open squares: Patients receiving 1 or 2 injections, often small test doses, of arsenical drug. Shaded squares: Patients receiving 3 or more therapeutic doses of arsenical drug.

arsenical drugs. There was no evidence that the dermatitis produced by one of these arsenicals differed in severity or duration from the group as a whole. It is clear, however, that dosage and repeated injections may affect the duration of the reaction. Figure 3 shows that exfoliative dermatitis following only 1 or 2 injections of arsenic (often small doses because of suspected hypersensitivity) was frequently quite brief, in contrast to the generally more prolonged course in patients who received 3 or more therapeutic doses of an arsenical drug. Three deaths occurred in this latter group of 36 patients.

The dermatitis in the patients treated with BAL was probably of somewhat greater average severity than in the control group. The patients were admitted to the hospital at about the same time (average of both series was the 10th day).

In the group treated with BAL, there were a number of patients who recovered with unexpected rapidity (Figure 3). This difference from the control series is most evident in the patients who received 3 or more therapeutic doses of arsenic. On the other hand, the cases who received smaller and fewer doses might have recovered spontaneously at this time, and little can be said about the effect of BAL on the duration of the disease. No deaths occurred in the 15 patients who received BAL, although 3 deaths occurred in the control series of 49 patients and far higher mortality rates have been reported in patients with severe exfoliative dermatitis (8, 9).

SUMMARY AND CONCLUSIONS

Twenty-two cases of arsenical dermatitis have been treated with 2,3-dimercaptopropanol (BAL).

Seven of these patients with an intractable, localized dermatitis caused by diphenylamine chlorarsine improved within a few days after the inunction of BAL in ointment.

Fifteen cases of generalized, exfoliative dermatitis following the use of antisyphilitic arsenicals have been treated with inunction or injection of BAL. Symptomatic and objective improvement regularly followed the administration of BAL. The duration of the dermatitis in over $\frac{1}{2}$ of the patients treated with BAL was shorter than in a comparable group of patients who were not treated with BAL.

A mild recurrence of the dermatitis was frequent when treatment was not continued for at least 1 week. Six such relapses cleared quickly after reinstitution of therapy.

BAL in ointment was quite painful when applied to inflamed skin, while intramuscular injections were much less disturbing. No serious constitutional reaction to BAL was observed with the small doses employed.

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CLINICAL USES OF 2,3–DIMERCAPTOPROPANOL (BAL). VIII. THE EFFECT OF BAL ON THE EXCRETION OF ARSENIC IN ARSENICAL INTOXICATION [1]

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WITH THE TECHNICAL ASSISTANCE OF EMILY B. WATSON

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The toxic effect of arsenic on the living cell probably follows the combination of the arsenic with certain vital components of the cell (1 to 5, 8). This combination has been observed to be reversible *in vitro* (5 to 9). With the development of 2,3-dimercaptopropanol (BAL) by Peters, Stocken, and Thompson (8), the detoxification of arsenic by dissociation of the arsenic-cell complex seemed feasible in man, since BAL has the requisite affinity for arsenic without undue toxicity (8 to 10). BAL was therefore administered in ointment, and later by injection, in the treatment of arsenical intoxication (11, 12).

An increased elimination of arsenic from the body during BAL treatment would probably reflect the release of a significant amount of arsenic from the cells (8, 10). To test this point, the urinary excretion of arsenic has been followed before and during treatment with BAL in 18 patients in this clinic. Sixteen were suffering from a complication of arsenical therapy. The effect of BAL on these patients' course is described in another report (11). The other 2 patients, who had received mapharsen without untoward reaction, were studied to determine the effect of BAL administered 2 and 3 days after an injection of the arsenical drug. The effect of BAL on the fecal excretion of arsenic was not followed, since the brief control period and the illness of the patients prevented the collection of accurately timed specimens.

METHODS

Arsenic in the urine was determined by the method of Magnuson and Watson (13).

For inunction, BAL was added in 5 or 10 per cent concentration to an ointment containing benzyl benzoate in an oily base. This ointment was devised by Mr. R. S. Fuqua and tested by Dr. W. F. Hughes, Jr., in Lewisite burns of the eye (14). BAL was given intramuscularly in 5 or 10 per cent concentration in peanut oil and benzyl benzoate (10).

Urinary sulfur was measured by the gravimetric procedures of Folin and Benedict (17).

The titration of urine with iodine was performed at a strongly acid reaction. Approximately 1 volume of concentrated HCl was added to 5 volumes of urine, and the mixture quickly titrated with 0.01 N iodine solution. Speed of titration was emphasized, since the urine may take up excess iodine slowly. A trace of freshly prepared starch solution was used as an indicator. Highly concentrated urine gave a poor end-point, and was diluted suitably. Duplicate or triplicate measurements were made. BAL added to urine could be measured by the procedure, but the reaction is obviously not specific for BAL. The results are presented to indicate the increment of reducing substances, including reduced sulfur, which accompanies the use of BAL.

RESULTS

Eight patients with arsenical dermatitis were given BAL by inunction or injection. In every case, there was an increase in arsenic excretion following the first course of BAL treatment (Table I). Four patients received a second course of treatment, with an increase in urinary arsenic in each case (Table I, Figures 1, 2, and 3). One of these patients, after responding to 2 series of BAL injections, showed an increased excretion of arsenic following a 3-days course of inunction with BAL in ointment, but gave no clear response to a subsequent single, large inunction (Figure 3). The total courses of treatment numbered 14 in 8 patients, with increased arsenic excretion in 13 trials in 8 patients.

In contrast to the findings in dermatitis, only 3 of the 6 patients with jaundice showed an increased excretion of arsenic after a course of

TABLE I

Effect of BAL on urinary excretion of arsenic

Diagnosis	Patient	Arsenical Drug	Arsenical Days As-BAL	BAL Route	BAL Daily dose (grams)	Days before BAL 4	3	2	1	Days after BAL 1	2	3	4	5	6	7	8	9	10
Dermatitis	M.M.	DM		O	0.5				26	30	121	43							
	G.G.	DM		O	0.5				56	137	57	35							
	E.H.	Neo	22	O	1.0		350	324	221	383	540	488	333	225	231	302	315		
	R.S.	Neo	11	O	0.5	229	181	208	128+	166	159	189	223	147	85	42	64	40	54
	R.S.	Neo	21	O	0.5		62	54	67	91	73	111	90	66	54				
	H.T.	Neo	16	O	0.5		200	71	248	592	579	415	565	372	451	300	401	187	245
	H.T.	Neo	35	O	0.5				155	170	275	221	809	309	207	94	143		181
	V.H.	Neo	16	I	0.3		248	291+	528	506	757	1408	292	372	230				
	V.H.	Neo	23	I	0.15			308	270	373	480	430	230		224				
	V.H.	Neo	28	O	0.25				232	241	240	349	224						
	V.H.	Neo	34	O	1.0				259	225	256	98+	195						
	H.C.	Neo	12	I	0.1		189	320	270	592	400	110+	230	60+					
	H.C.	Neo	21	I	0.15			100	100	155	166	225	106						
	H.B.	Map	10	I	0.4		40	48	21	77	50	11		48					
	M.G.	Map	5	O	1.0	1990	1850	1365	1325	688	390	213	210	175	94	77			
	E.B.	Ars	30	I	0.3		336	452	320	320	293	388	314	270	264	256	177		
	L.C.	Map	8	I	0.3		120	171	165	320	400	388	314	270	309	372	405		
	B.F.	Sil	21	I	0.5		212	226	407	186	298	388	102	124					
	A.H.	Map	95	I	0.3		41	56	71	62	102	22	66	62	38	40			
	E.P.	Map	9	I	0.3		164	137	171	128	176	143							
Blood dyscrasia	E.A.	Neo	4	O	0.5				1325	793	877	544	569	647	309	372			
	E.W.	Neo	6	I	0.5			498	520+	512	415	388	314	270					
Jaundice	J.F.	Map	2	I	0.2	46	16	66	87	542	251	151	102	124					
	C.S.	Map	3	I	0.5	945	755	643	324	454	206	143	66	62	38	40			

Ars. = Arsphenamine
Sil. = Silver arsphenamine
Neo. = Neoarsphenamine
Map. = Mapharsen

O = In ointment
I = By intramuscular injection

+ = Incomplete specimen
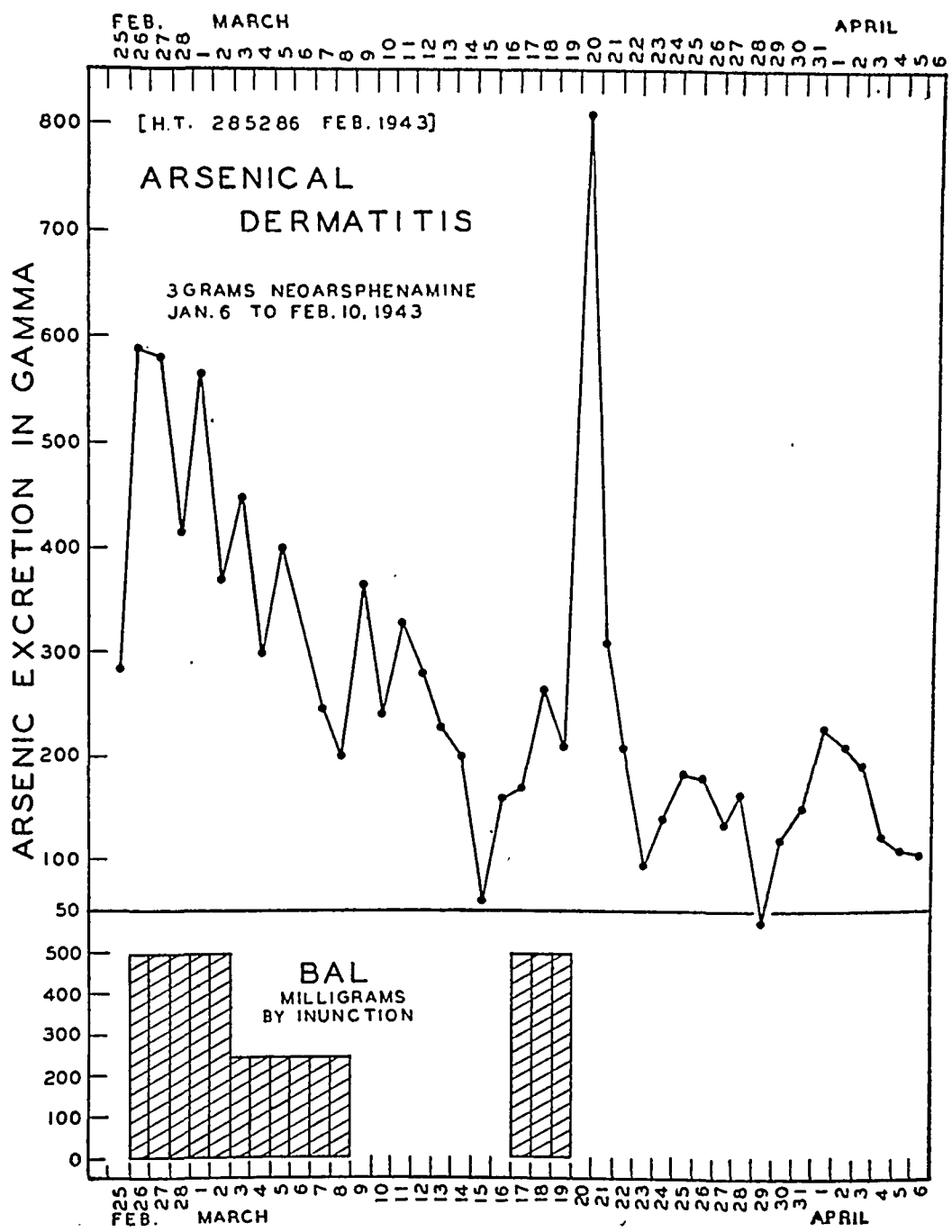
Days of Treatment Underlined

FIG. 1. EFFECT OF TWO COURSES OF INUNCTION OF BAL OINTMENT ON DAILY
ARSENIC EXCRETION IN ARSENICAL DERMATITIS (PATIENT H. T.)

BAL treatment. In the whole series of cases, all of the obvious failures of increased excretion were in cases of jaundice occurring during antisyphilitic treatment.

The 2 patients with agranulocytosis and thrombocytopenia entered the hospital within a few days of the last injection of arsenic, when the rapidly decreasing excretion of arsenic made the effect of BAL treatment difficult to demonstrate. In 1 patient (E. W.) there was possibly a small in-

crease in urinary arsenic after treatment, but in the other (E. A.), no effect was evident. In order to study a similar situation with adequate control observations in patients without a pressing need of treatment, 2 men under antisyphilitic treatment were given BAL by intramuscular injection 2 or 3 days after the last arsenical injection. The results (Figure 5) were very similar to those seen in the patients with blood dyscrasia. During the period of rapidly decreasing arsenic
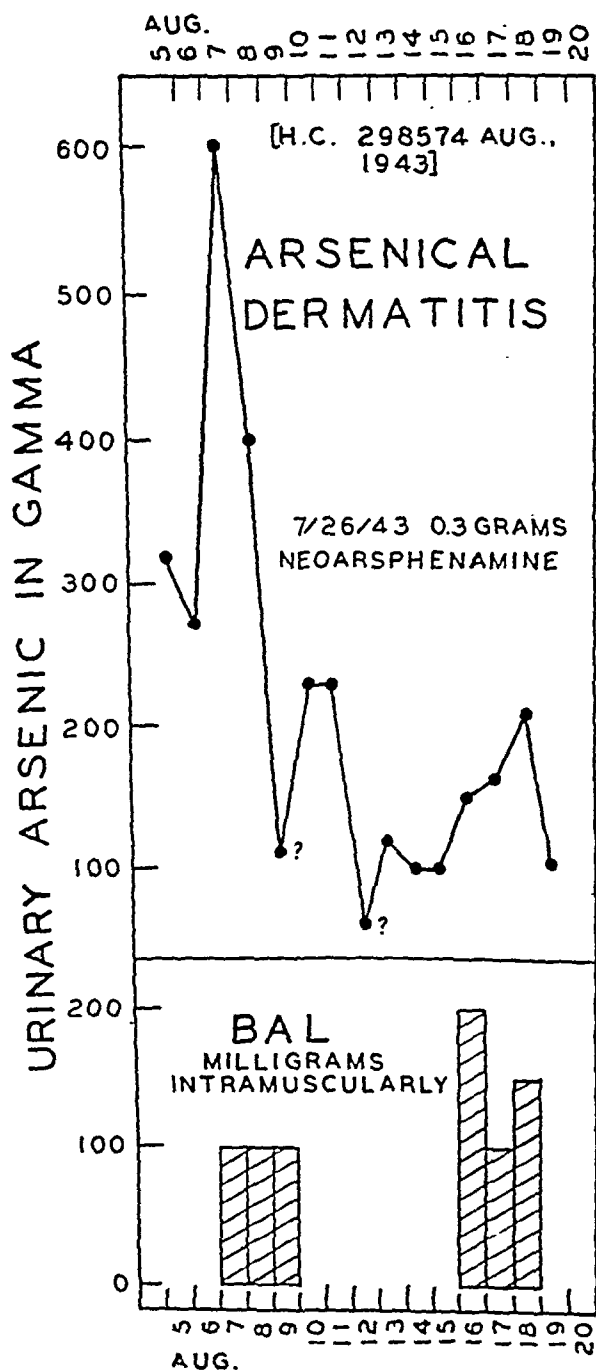
FIG. 2. EFFECT OF TWO SERIES OF INTRAMUSCULAR INJECTIONS OF BAL ON DAILY ARSENIC EXCRETION IN ARSENICAL DERMATITIS (PATIENT H. C.)

excretion, little or no increase of urinary arsenic was observed after BAL, but a rapid fall in arsenic excretion might be noted at the end of treatment.

The effect of the route of administration of BAL on the excretion of arsenic cannot be evaluated because of the small number and diversity of the cases. It is evident that either inunction or injection of BAL is generally quite effective. The dosage by inunction was usually larger.

A few experiments were undertaken to observe the relation between urinary sulfur and arsenic during the administration of BAL. In 3 patients, excretion of organic sulfur increased after BAL (Figures 4 and 5). In each case, the excretion of arsenic was affected to a greater or less degree by the administration of BAL, and the change coincided with an increase of organic sulfur in the urine. The reverse effect could not be demonstrated in 2 cases (Figure 5) in which an injection of mapharsen did not appreciably affect the excretion of organic sulfur.

As a rough measurement of the addition of reduced sulfur to the urine after the administration of BAL, the iodine uptake of strongly acidified urine was measured. This quantity, recorded as m. eq. of iodine per day, increased after BAL treatment and closely followed the changes of arsenic excretion in 2 cases (Figures 4 and 6).

DISCUSSION

The consistent increase in arsenic excretion after BAL treatment of arsenical dermatitis corresponds with the good clinical response of these patients (11). Although it is apparent that some arsenic is removed by the BAL, the excretion of arsenic continues at a lower level after the completion of treatment. There are reasons to believe that the arsenic removed by BAL is that which is most damaging. The combination of the skin with highly toxic arsenicals has been demonstrated to ·be reversible by BAL (8, 9). BAL has been shown to restore cellular ferments of the skin inactivated by toxic arsenicals (8, 9). Furthermore, the most toxic arsenical compounds, which are bound most firmly to the living cell, show the greatest stimulation of excretion after BAL (3, 10). These observations suggest that the increase in urinary arsenic after BAL treatment reflects the release of a toxic arsenical from the skin, with consequent improvement of the dermatitis.

No such regular improvement is evident, either
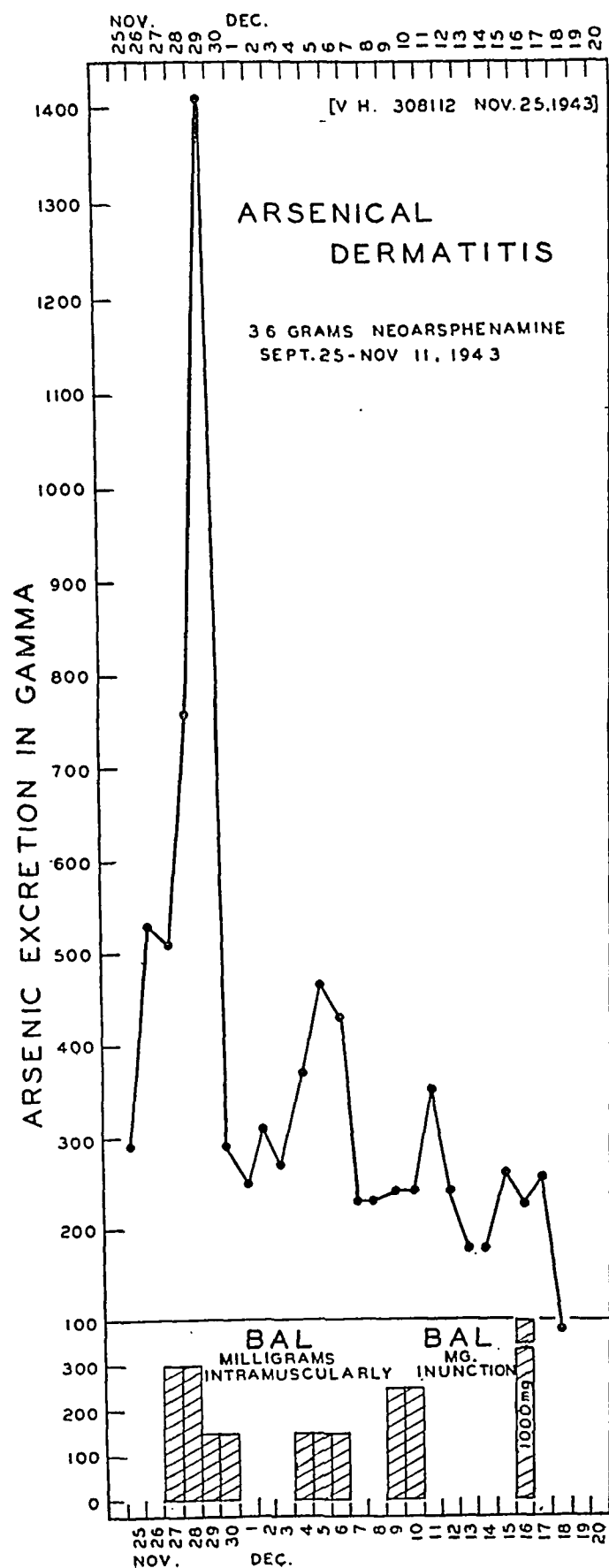
ARSENIC

I₂ TITER

SULFUR

BAL
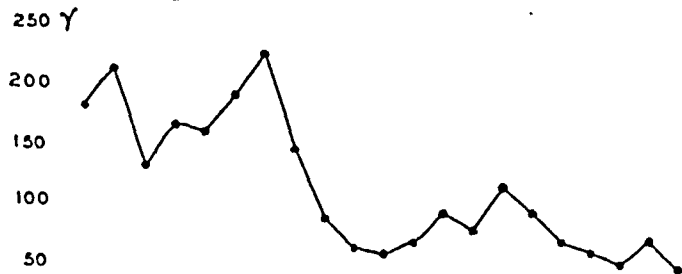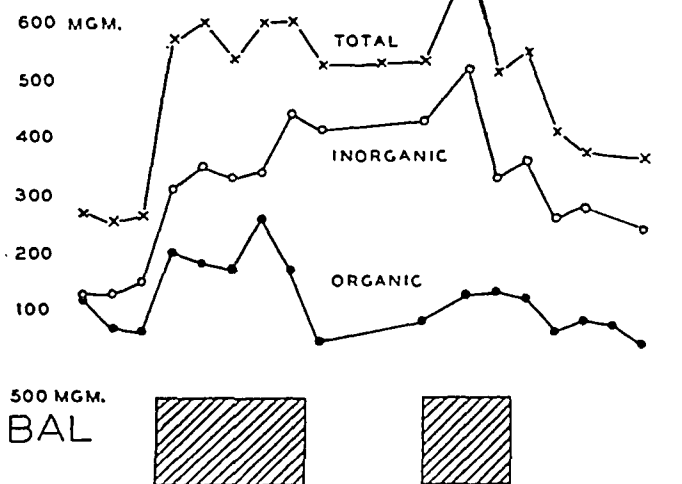
FIG. 4. DAILY URINARY EXCRETION OF ARSENIC, IO-
DINE-TITRABLE REDUCING SUBSTANCES, AND SULFUR (OR-
GANIC, INORGANIC, AND TOTAL, INCLUDING ETHEREAL)
DURING TWO COURSES OF TREATMENT OF ARSENICAL
DERMATITIS WITH INUNCTIONS OF BAL (PATIENT R. S.)

clinically or in the excretion of arsenic after BAL
treatment, of the jaundiced patients. The arsenic
excretion was unchanged in 3 cases, and moder-

FIG. 3. EFFECT OF BAL ON DAILY ARSENIC EXCRE-
TION IN A PATIENT WITH ARSENICAL DERMATITIS (PA-
TIENT V. H.)

The patient received 2 courses of intramuscular injec-
tions of BAL. Subsequently, she was given a brief
course of inunction with BAL in ointment and finally a
single large inunction.

ately increased in the other 3. It is difficult to assess the etiologic rôle of arsenic in these cases, since intercurrent hepatitis would produce much the same picture (15). Moreover, the type of jaundice produced by arsenical drugs varies (16), and BAL might not be expected to affect each type equally. The phosphatase activity as a measure of biliary obstruction shows no correlation with the response to BAL.

The increased excretion of organic sulfur and reducing substances after the administration of BAL coincides with the increase in urinary arsenic. Considering the large excess of sulfur, it seems improbable that an increased urinary arsenic could affect appreciably the excretion of organic sulfur, and no such effect is evident. These observations support the idea that the administration of BAL promotes the excretion of arsenic by supplying an excess of material with which arsenic forms stable compounds.
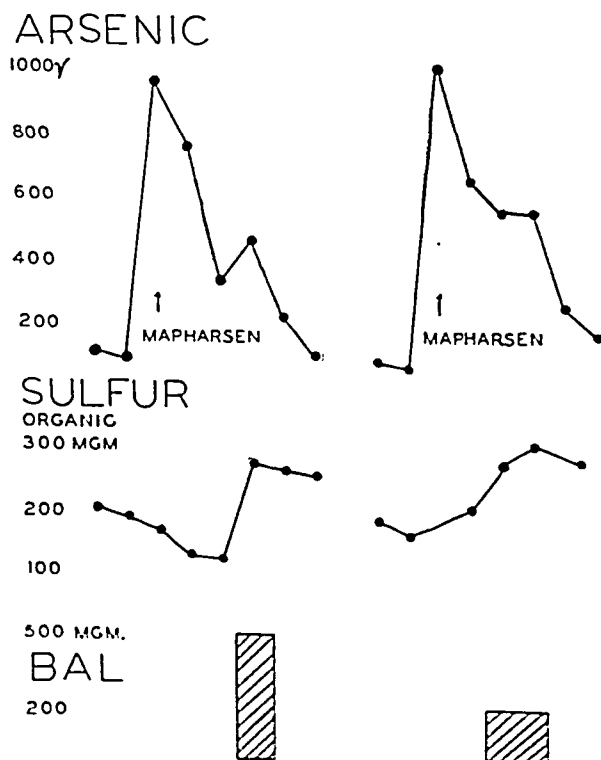
Fig. 5. Daily Urinary Excretion of Arsenic and Organic Sulfur in Two Patients (C. S., Left, and J. F., Right) Receiving Mapharsan Followed After 2 to 3 Days by Intramuscular Injection of BAL
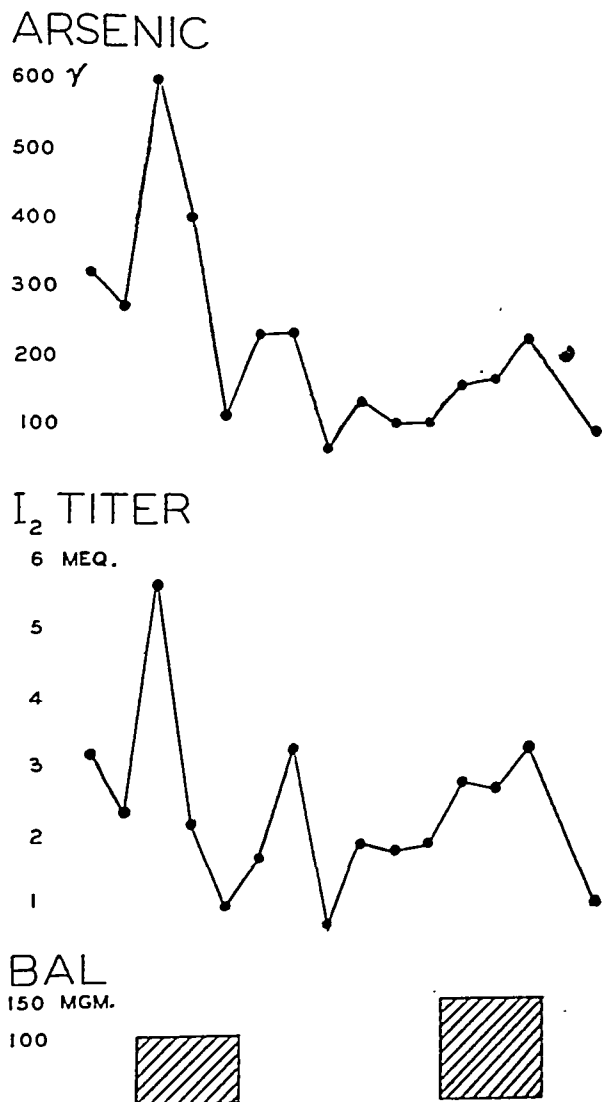
Fig. 6. Daily Urinary Excretion of Arsenic and Iodine-titrable Reducing Substances During Two Courses of Treatment of Arsenical Dermatitis with Intramuscular Injections of BAL (Patient H. C.)

SUMMARY

The urinary arsenic has been studied before and after BAL therapy in 16 patients with arsenical intoxication, and in 2 patients receiving arsenical treatment without reaction. Of 24 courses of BAL administration, 16 were followed by a definite increase in arsenic excretion, 4 by a possible increase, and 4 by no increase. On no occasion was there a decreased excretion of arsenic during BAL treatment.

The increase in urinary arsenic after BAL appears to be more consistent in patients with the arsenical dermatitis than in hepatitis, suggesting a correlation with the greater efficacy of BAL in the treatment of arsenical dermatitis.

The increases of urinary organic sulfur and reducing substances coincide with the increased urinary arsenic after BAL administration, lending support to the idea that the excretion of BAL or a related substance is associated with the increased arsenic excretion.

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CLINICAL USES OF 2,3–DIMERCAPTOPROPANOL (BAL). IX. THE TREATMENT OF LEWISITE BURNS OF THE EYE WITH BAL [1]

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Exposure of the eye to relatively small quantities of Lewisite liquid or vapor produces a devastating ocular lesion. As will be demonstrated below, the progressive nature of such a burn is caused by the arsenical component of this war gas. To decontaminate the tissues of arsenic after exposure to Lewisite, the English workers (1) synthesized 2,3-dimercaptopropanol (CH_2-SH–CHSH–CH_2OH), the so-called BAL. The work pursued in our laboratories was devoted to the determination of the optimum conditions for the use of this antidote, its mode of action, and limitations. These experiments will be discussed under the following headings: (*1*) certain clinical and pathologic characteristics of Lewisite burns of the eye relating to mode of action, rate of penetration, and the time at which irreversible histological changes first develop; (*2*) the rate of penetration and persistence of arsenic in the tissues after Lewisite burns, in relation to the decontaminating action of BAL; and (*3*) the toxicity and therapeutic efficacy of BAL.

Most of the details of the experiments and a description of the effects of Lewisite on the eye will be reported elsewhere (2). It is the purpose of this communication to summarize the evidence that BAL penetrates rapidly into Lewisite-burned eyes, and competes successfully with toxic arsenical material within the tissues.

Early changes in the ocular tissues after exposure to Lewisite

During the course of this work, more than 600 rabbit eyes have been exposed to Lewisite. The clinical courses of liquid or vapor burns are essentially similar, and are characterized by rapid tissue necrosis, marked conjunctival and corneal

edema, and intense exudation. Immediately on contact with the moist surface of the eye, Lewisite hydrolyzes with the production of an arsine-oxide and hydrochloric acid:

$$ClCH = CHAsCl_2 + H_2O =$$
$$ClCH = CHAsO + 2\ HCl,$$
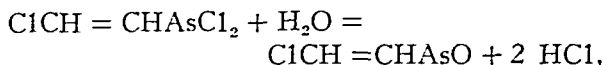
At the site of contact with the cornea, sufficient hydrochloric acid is liberated to reduce the local pH below 1.3 as tested by thymol blue. Acidity of this degree is sufficient to produce a localized superficial opacity of the cornea (3), and subsequent treatment with BAL has no effect on this acid component of the Lewisite burn. All of the later progressive characteristics of a Lewisite burn can be produced by the instillation of a dilute solution of Lewisite-oxide, and are therefore attributable to the arsenic component of this war gas. It was also found that a number of other compounds containing trivalent arsenic (*e.g.*, sodium meta-arsenite, phenyl arsine oxide, and mapharsen) were more toxic on intracorneal injection of dilute solutions, than compounds containing pentavalent arsenic (*e.g.*, sodium arsenate and tryparsamide).

Within 10 minutes after exposure to Lewisite and after 30 minutes, histological evidence of damage appears in all tissues of the anterior ocular segment, indicating deep penetration and rapid necrotizing action of this toxic arsenical (Figures 1 and 2).

Rate of penetration and persistence of arsenic in the eyes of rabbits burned with Lewisite

The rate of penetration and persistence of toxic arsenical material in the cornea and aqueous following exposure to Lewisite were determined, in order to establish time limits during which either a 100 per cent efficient surface decontaminating agent, or an ideal penetrating agent, would be effective, and to study the effect of treatment with
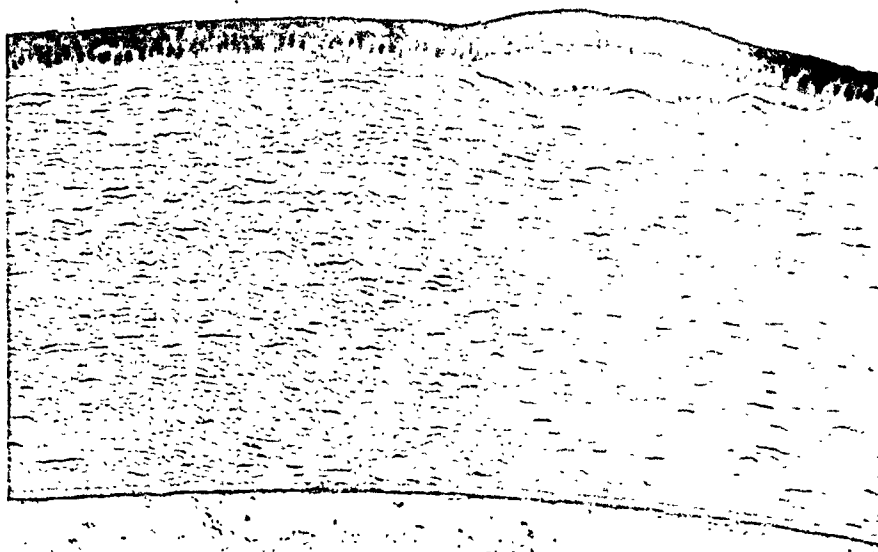
Fig. 1. Section of Rabbit's Cornea, 10 Minutes After Exposure for 30 Seconds to Saturated Lewisite Vapor at 22° C.
Early changes have taken place in the corneal epithelium which is partially loosened from the underlying stroma. The corneal endothelium is disintegrated, and serum is present in the anterior chamber.

BAL on the persistence of arsenic within the tissues.

MATERIAL AND METHODS

Rabbit corneas were exposed to Lewisite, either by the instillation of 0.1 mgm. of the liquid onto the center of the cornea, or by exposure of the proptosed eye for 30 seconds at 22° C. to saturated vapor in a Scholz vapor chamber (4). The lids were then closed within 30 seconds after exposure. At varying intervals thereafter, the corneas were excised or the aqueous was withdrawn and analyzed for arsenic, according to the method of Chaney and Magnuson (5). With this method, "blank" control tissues may show as much as 0.5 micrograms of arsenic. The experimental error for determination of these small quantities of arsenic is about 10 per cent, but any value over 1.0 microgram indicates a definite presence of arsenic. Since any organic combination of arsenic is converted to inorganic arsenic by this method, the presence of BAL does not interfere with the sensitivity of the test (6).

A second series of experiments was performed to determine the persistence of toxic material on the surface of the cornea, in the cornea, or in the aqueous. Residual toxic material in such burned eyes was detected by transfers to normal rabbit corneas, either by direct contact of the corneal surfaces, by the intracorneal injection of pressed-juice extracts of the corneal stroma, or by the intracorneal injection of aqueous. The reaction in the normal eye produced by the transferred material was then graded according to a numerical evaluation of the symptoms produced, thus giving an index of the toxicity of the material tested.

RESULTS

Within 2 to 4 minutes after the instillation of liquid Lewisite into the eye followed by closure of the lids, little or no residual toxic material or arsenic remained on the surface of the cornea.

Two minutes after exposure to Lewisite vapor or liquid, from 3.0 to 4.6 micrograms of arsenic were demonstrated within the corneal substance (Table I). The amount of arsenic diminished greatly within the next 30 minutes, and after 11 hours showed only traces of arsenic irregularly, up to 4 days. The urinary excretion of arsenic after the systemic administration of toxic arsenicals has been reported to be very slow, indicating a rather firm attachment of arsenic to the tissues (7, 8). Corneal juice continued to be toxic after 1 hour, but was non-toxic after 26 hours (Table II).

Arsenic penetrated into the anterior chamber within 1½ minutes after the application of liquid
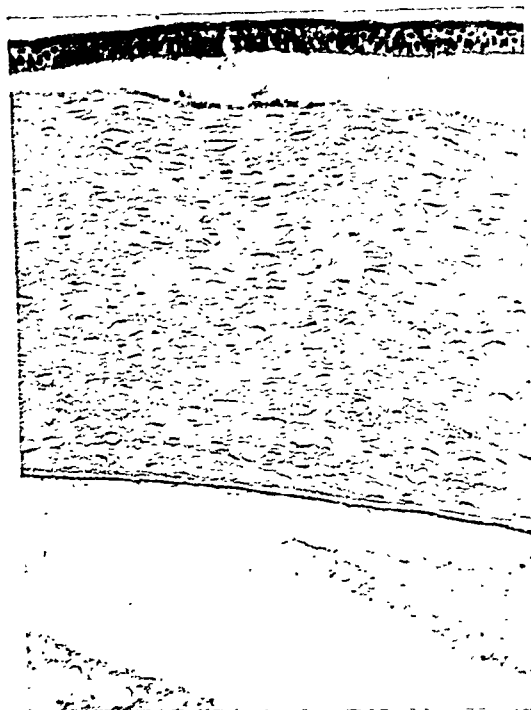
FIG. 2. SECTION OF RABBIT'S CORNEA, 30 MINUTES
AFTER EXPOSURE TO LEWISITE VAPOR, SHOWING CHANGES
IN THE CELLS OF THE CORNEAL STROMA

Lewisite to the cornea, and disappeared within 30 minutes after exposure (Table III). The aqueous was found to be toxic at 2 minutes and 10 minutes, but had become non-toxic when tested at 30 minutes.

Surface decontamination after exposure to liquid Lewisite. In view of the evidence above, that Lewisite rapidly disappears from the surface of the cornea and penetrates into the cornea and aqueous, little or no therapeutic effect would be expected from the use of neutralizing agents which do not penetrate into the ocular tissues. Such was found to be the case with 3 types of agents tried: (1) saline irrigation, (2) an iodine-containing solution, so-called "Box I," which will inactivate Lewisite in 15 seconds *in vitro,*[2] and (3)

[2] This solution was devised by Dr. Leslie Hellerman as a method for the continuous regeneration of a limited low concentration of iodine:

NaI   200 mgm. per 100 ml.
Phosphate buffer pH 7   M/10
Sodium iodoxybenzoate in large excess
Solvent: 50 per cent triacetin, 22 per cent alcohol, and 28 per cent water.

TABLE I

Microchemical analysis of arsenic in cornea following Lewisite burns of the rabbit's eye

Dose of Lewisite	Time after application	Gamma of arsenic
Controls (no Lewisite)		0. to 0.5
0.1 mgm. liquid	2 min.	6.5
		4.1
		6.5
		4.2
		3.7
		2.7
		4.7
	30 min.	1.2
		1.6
	1 hr.	0.7
		2.9
	2 hrs.	1.3
	4 hrs.	1.8
	11 hrs.	0.5
	18 hrs.	0.
	24 hrs.	1.7
		0.5
		0.5
	2 days	0
	4 days	0.9
Saturated vapor 22°C. for 30 secs.	2 min.	2.7
		2.9
		3.3
	30 min.	1.2
	1 hr.	1.9
	4 hrs.	1.3
	24 hrs.	0.8

hydrogen peroxide (Table IV). In these experiments, the technique for the production and grading of the standard lesion was that described below for the BAL treatment experiments.

The treatment of Lewisite burns of the eye with BAL

Since Lewisite penetrates through the cornea into the aqueous within 2 minutes after exposure, and produces irreversible changes in the tissues

TABLE II

Toxicity of corneal juice after instillation of 0.1 mgm. of liquid Lewisite into a rabbit's eye

Time after instillation of Lewisite	Maximum ocular reaction produced by intracorneal injection of corneal juice into normal rabbit's eye
2 min.	47
30 min.	30
1 hour	17
26 hours	2
27 hours	2
4 days	2 (2 injections)

TABLE III

Microchemical analysis of arsenic in aqueous following the instillation of 0.1 mgm. of Lewisite on the rabbit's cornea

Time after instillation of Lewisite	Gamma of arsenic
Aqueous control (no Lewisite)	0.4 (blank of method = 0.0 to 0.5 gamma)
Needle control (for surface contamination in withdrawing aqueous)	0.2
1 min.	0.5
1.5 min.	2.2
5 min.	2.8
10 min.	3.2 (2 specimens)
20 min.	3.2
30 min.	0
1 hr.	0
2 hrs.	0.5
11 hrs.	0.9

of the anterior ocular segment soon after 10 minutes have elapsed, a successful decontaminating agent must have the capacity to penetrate rapidly and to compete successfully for both free and combined arsenic within the tissues. To obtain maximum therapeutic effect from the local use of BAL after exposure to Lewisite, the following variables were studied: (*1*) local toxicity of BAL for both normal and Lewisite-burned eye, (*2*) optimum concentrations of BAL, and techniques of application to the eye, (*3*) the time limits during which treatment is efficacious, and (*4*) species differences in toxicity and therapeutic effectiveness of BAL.

MATERIALS AND METHODS

Preliminary experiments were devoted to the production of a reproducible and uniform standard lesion by Lewisite, the technique and dosage to be used on treatment experiments. In general, it is necessary that the

TABLE IV

Surface decontamination of the rabbit's eye 2 minutes after the instillation of 0.1 mgm. of Lewisite

Number of eyes	Decontaminating agent	Maximum ocular reaction	Final corneal opacity	Days
		per cent	*per cent*	
3	100 ml. saline irrigation	72 (SD =10)*	75 (SD =19)	7
3	"Box I" (iodine solution)	66 (SD =3)	69 (SD =2)	7
6	2 per cent H₂O₂ (3 drops)	69 (SD =4)	65 (SD =6)	7
3	Blood catalase alternating with 5 per cent H₂O₂ every 5 secs. for 1 min.	63 (SD =20)	50 (SD =30)	7
42	No treatment	74 (SD =8)	69 (SD =14)	7

* SD = Standard deviation of the mean.

lesion be sufficiently severe to guarantee reproducibility, and yet an excessive dosage should be avoided which might mask the efficacy of a therapeutic agent of low potency. Such a threshold lesion was produced by the instillation of 0.1 mgm. of liquid Lewisite directly from a micrometer syringe and No. 26 blunt needle either on the limbus or center of the cornea. A somewhat less severe but consistent lesion was produced by exposure of the proptosed eye to saturated Lewisite vapor at 22 to 24° C. for 30 seconds. The severity of the lesion produced was evaluated numerically by grading the important clinical symptoms elicited by the toxic agent. In order to obtain single values which could be used for statistical comparisons, the acuteness of the reaction was estimated by adding the maximum values for each symptom over the course of observation and conversion of the total, to a percentage figure, the so-called "maximum ocular reaction." An index of persistent corneal damage was obtained by adding the values of the corneal symptoms on the last day of observation, a total of 24 points or corneal perforation representing a 100 per cent lesion.

Preliminary treatment experiments were made with a sample of BAL obtained in the spring of 1942 from Dr. Peters' Laboratory at Oxford. Very little difference in toxicity or therapeutic efficacy could be detected between the British and American (NDR 133 Q-Z) samples of BAL. The American sample appeared to be slightly more toxic at higher concentrations, and more effective at lower concentrations, but the British sample was older at the time of comparison. Subsequent experience with later samples of American BAL has shown results comparable to those reported here for the earlier sample NDR 133 Q-Z.

Suitable solvents for BAL were found in propylene glycol, ethylene glycol, redistilled thiodiglycol and triacetin. BAL is sparingly soluble and deteriorates rapidly in water, but is effective therapeutically in aqueous solution. Because of many practical advantages in applying ointments to the eye, a BAL ointment was devised and prepared by Mr. Robert S. Fuqua, Chief Pharmacist of the Johns Hopkins Hospital, for use in these experiments.

	per cent
BAL	5 to 10
Benzyl benzoate	5
Peanut oil	20
Absorbent base	20
Cetyl alcohol	10
Glycerin monostearate	14
Liq. white petrolatum	21

This ointment was readily miscible with the tears of the eye, and within a minute after instillation, became semifluid. It was non-toxic for the normal rabbit's and monkey's eyes, and showed therapeutic efficacy comparable to that of BAL in glycol solution.

Local toxicity of BAL. The tolerance of the normal cornea for BAL was determined on rabbits, monkeys and partially for humans (Table V).

TABLE V

Tolerance of the normal eye to 0.1 ml. of BAL solutions

Species	BAL	Solvent	Maximum ocular reaction	Final corneal opacity	Days
	per cent		*per cent*	*per cent*	
	50	Propylene Glycol	67	67	7
Rabbit	35	P.G.	40	13	7
	25	P.G.	20	0	2
	15	P.G.	20	0	1
	10	P.G.	5	0	0
	10	Ointment	2	0	0
	5	P.G.	2	5	0
	3	P.G.	5	0	0
	3	Water	2	0	0
	50	P.G.	42	38	7
Monkey	35	P.G.	37	21	6
	25	P.G.	22	0	4
	15	P.G.	0	0	0
	10	P.G.	2	0	0
	10	Ointment	0	0	0
	5	P.G.	0	0	0
Human					
2 eyes	10	P.G.	7	0	0
3 eyes	5	P.G.	5	0	0

Toxic concentrations of BAL produced a sheet-like opacification of the superficial layers of the cornea which, if not unduly severe, sloughed off within a few days leaving clear stroma beneath. Rabbits, monkeys, and humans tolerated single instillations of BAL of concentrations from 3 to 10 per cent. BAL is temporarily irritating to the human eye, and produces an immediate stinging sensation, followed by blepharospasm, epiphora and conjunctival congestion.

Since an ocular lesion is almost completely prevented by the use of 2 drops of 5 per cent BAL within 2 minutes after exposure to Lewisite (see below), the more severe reactions which follow the use of higher concentrations must be attributed to the toxic action of the therapeutic agent. This damage produced by BAL can be distinguished clinically in an eye previously burned with Lewisite. It is noteworthy that the reactions produced by concentrations of BAL over 5 per cent in eyes previously exposed to Lewisite were more severe than those produced by BAL on the normal cornea (Figure 3). This suggests that Lewisite lowers the tolerance of the cornea for BAL.

Penetrability of BAL. Direct iodometric titrations of the aqueous were made by Adler and his colleagues (9), who found an appreciable increase in the amount of sulfhydril-containing material 5 minutes after the instillation of BAL drops, or ointments, into the rabbit's eye. Such rapid penetrability of BAL is also demonstrated indirectly by its therapeutic effect on Lewisite-burned eyes.

Optimum concentrations of BAL and techniques of application in the treatment of Lewisite burns. Although a single instillation of dilutions of BAL ranging from 25 per cent to 1 per cent prevented the development of any corneal opacity lasting more than 8 days, the use of 5 per cent BAL was
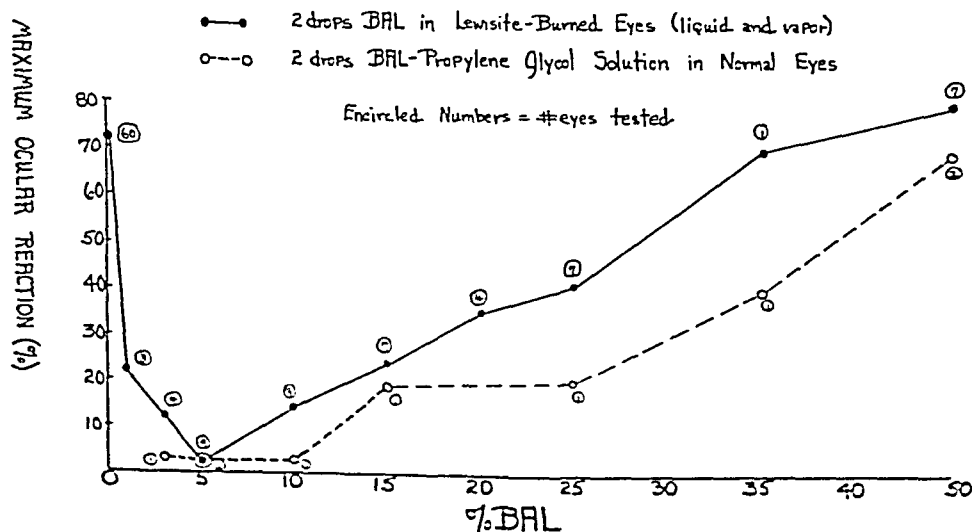

FIG. 3. EFFECT OF VARIATION IN CONCENTRATION OF BAL ON REACTIONS IN NORMAL AND LEWISITE-BURNED RABBIT EYES

found to be the optimum concentration which regularly produced spectacular cures of Lewisite burns (Table VI and Figures 4 and 5). Repeated in-

Effect of variation in concentration of BAL in the treatment of Lewisite burns of the rabbit's eye*

BAL solution	Number of eyes	Maximum ocular reaction	Final corneal opacity	Number of days followup
per cent		*per cent*	*per cent*	
25	1	45	0	6
20	3	37	0	6
15	4	23	0	4
10	3	11	0	2
5	5	4	0	1
3	2	12	0	2
1	1	20	0	2
Untreated**	42	74 (SD = 8)***	69 (SD = 14)	7

* Instillation of 2 drops of BAL in propylene glycol 2 minutes after application of 0.1 mgm. of liquid Lewisite.
** Instillation of 2 drops of propylene glycol has no influence on the severity of the Lewisite lesion.
*** SD = Standard Deviation of the Mean.

stillations of BAL did not result in any additional therapeutic benefit. The use of 5 per cent BAL ointment was found to be at least equally as effective as glycol solutions of BAL in the treatment of both liquid and vapor burns. BAL was also found to be effective in the treatment of Lewisite burns of the monkey's eye. The effectiveness of BAL against Lewisite has also been demonstrated in many experiments by others (9 to 12).

Time limits during which BAL is effective. The instillation of BAL solution, or ointment, within 2 minutes after the end of exposure to Lewisite usually prevents the development of any significant conjunctival or corneal reaction. If treatment is delayed for 5 minutes, a transitory conjunctival and corneal reaction ensues and lasts a few days. Treatment instituted 10 minutes after exposure is definitely less effective, a mild to moderate corneal opacity persisting at the end of 7 days. The use of BAL within the first half hour lessens the severity of the ocular lesion when compared to control eyes, but permanent damage to the eye remains.

These findings are in agreement with the fact that irreversible histological changes are already detectable in the corneal stroma within 10 minutes

after exposure to Lewisite, and are quite pronounced after 30 minutes. Also at the end of 30 minutes, some of the arsenic has already disappeared from the tissues, and so the benefit derived from decontaminating is diminished.

Persistence of arsenic in the cornea after the application of BAL

The quantity of arsenic remaining in the cornea at stated intervals after a standard exposure to Lewisite vapor was determined both for eyes treated with BAL, and for untreated controls (Table VII). Whereas a trace of arsenic was

Influence of BAL on the rate of disappearance of arsenic from the rabbit's cornea after Lewisite vapor burns

Specimen No.	BAL	Time of excision of cornea	Gamma of arsenic
1	0	2 min.	2.7
2			2.9
3			3.3
4		30 min.	1.2
5		1 hr.	1.9
6		4 hr.	1.3
7		24 hr.	0.8
8	2 drops of 5 per cent BAL 2 min. after exposure.	30 min.	0
9		4 hr.	0
10		24 hr.	0

demonstrated in the untreated corneas after 24 hours, those corneas treated with 5 per cent BAL showed no residual arsenic after 30 minutes. Thus the use of BAL facilitates the disappearance of arsenic from the tissues, probably by competing favorably with arsenic reversibly bound to the tissue components.

Intravenous treatment of Lewisite burns of the eye with BAL

Toxic doses of English BAL were administered intravenously to 2 rabbits in which 0.1 mgm. Lewisite had previously been instilled into the eyes. The conjunctival and, to some extent, the corneal reaction in these 2 animals was less than the average ocular reaction in control animals.

SUMMARY AND CONCLUSIONS

Exposure of the eye to small quantities of Lewisite liquid or vapor can produce a progressive

FIG. 4. BOTH EYES EXPOSED 24 HOURS PREVIOUSLY FOR 30 SECONDS TO SATURATED LEWISITE VAPOR AT 22° C.

Right eye treated 2 minutes after exposure with 0.1 ml. of 5 per cent BAL in propylene glycol. Left eye is untreated.
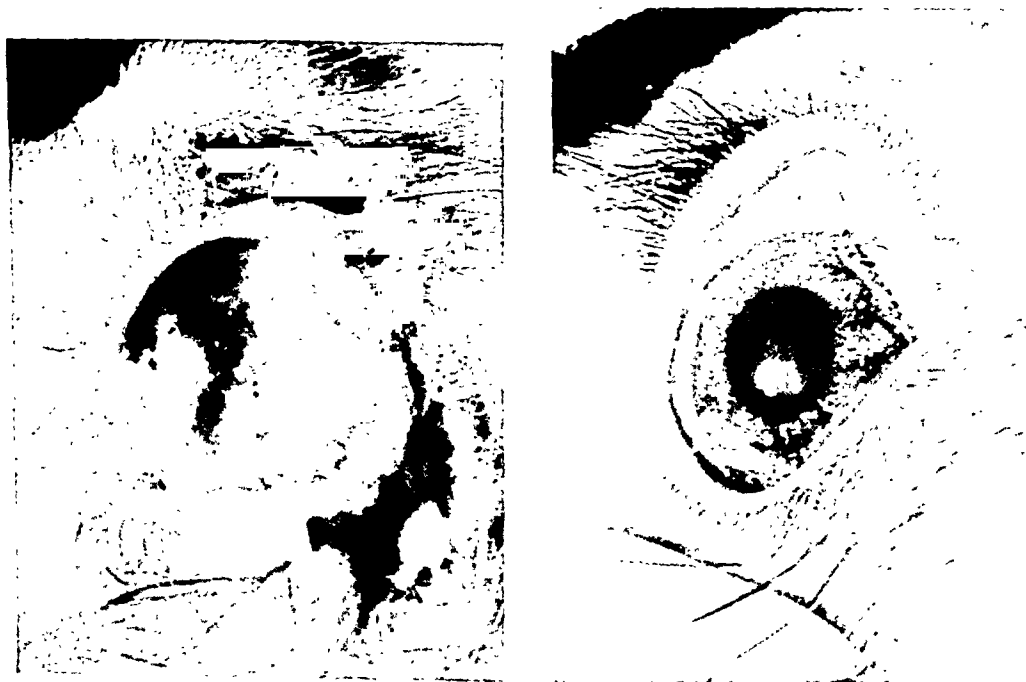

FIG. 5. EYES EXPOSED AND TREATED IN SAME MANNER AS THOSE SHOWN IN FIGURE 5, 4 DAYS AFTER EXPOSURE

lesion of the cornea characterized by rapid tissue necrosis, marked edema and intense exudation. Lewisite is immediately hydrolyzed at the site of contact with the moist surface of the eye, liberating hydrochloric acid sufficient to produce a superficial corneal opacity. The more destructive characteristics of Lewisite burns can be produced by a neutral solution of Lewisite-oxide containing the trivalent arsenic. Within 2 to 4 minutes after exposure to Lewisite followed by closure of the lids, all toxic arsenical material disappears from the surface of the cornea, and within 2 minutes can be demonstrated in the aqueous. Beginning at 10 minutes after exposure, and well marked at 30 minutes, irreversible histologic changes in the cornea can be detected.

A single instillation of 5 per cent BAL solution, or ointment, within 2 to 5 minutes after exposure to Lewisite effectively prevents the development of serious ocular lesions. This excellent therapeutic effect of BAL is due, in part at least, to its rapid penetration and withdrawal of toxic arsenical material from the tissues before irreversible histologic changes have developed.

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CLINICAL USES OF 2,3–DIMERCAPTOPROPANOL (BAL).   X. THE TREATMENT OF ACUTE SYSTEMIC MERCURY POISONING IN EXPERIMENTAL ANIMALS WITH BAL, THIOSORBITOL AND BAL GLUCOSIDE

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Although chemical agents were not employed as offensive weapons during the past war, nevertheless it was imperative to pursue vigorously investigations on the treatment of chemical warfare casualties. Inasmuch as a number of chemical warfare agents contain arsenic, attention was focused on the synthesis of compounds capable of antagonizing the local and systemic effects of arsenical vesicants. These investigations, initiated by the British, led to the synthesis of 2,3-dimercaptopropanol, which is now more familiarly known as BAL (British Anti-Lewisite). In addition to BAL, numerous other SH-containing compounds, including monothiols and dithiols of polyhydric alcohols, were synthesized by the American and British investigators. Among these were thiosorbitol, prepared at the du Pont de Nemours and Company laboratories, and the glucoside of BAL, first synthesized by Danielli and co-workers (1).

The historical background which led to the synthesis of BAL and its derivatives, and the basic contributions which have resulted from studies on the effect of mercaptans on the toxic action of arsenic and other heavy metals, have been summarized by Peters (2) and Waters and Stock (3). Pertinent to the background of the present study were the observations supporting the general hypothesis that heavy metals are toxic to biological systems because of their reaction with SH groups of the protein moiety of cellular enzymes to form mercaptides. That mercury shares in this action has been demonstrated by Barron and co-workers (3). Moreover, BAL is capable of reactivating enzyme systems poisoned by mercury,

a fact which both affords support to the theory of the mechanism of inactivation, and gives promise for the therapeutic efficacy of mercaptans in the treatment of mercury poisoning. The present report is concerned with the efficacy of BAL, BAL glucoside and thiosorbitol in the treatment of experimental, acute, systemic mercury poisoning in rabbits and dogs.

The reactions between mercaptans and Hg^{++} in vitro

The reactions between mercaptans and cationic mercury which occur *in vitro* are presumably indicative of the expected interaction of the two agents *in vivo*. A brief study of the chemical reactions between the various mercaptans and mercury was, therefore, undertaken.

Reactions with BAL. When solutions of $HgCl_2$ (0.1 M) were added to non-buffered, aqueous solutions of BAL (0.05 M) a copious, flocculent white precipitate formed. Titration with phenolphthalein as an indicator revealed the formation of 2 equivalents of H^+ for each mol of $HgCl_2$ added. The white precipitate was insoluble in alkali, but could be dissolved upon the addition of concentrated HCl. On the basis of analogy to the reactions of BAL with arsenicals (2,3) the complex (hereafter called Hg-BAL) presumably possessed the following structure:
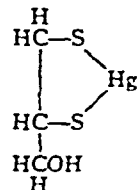
The complex was sufficiently dissociable so that the addition of neutral solutions of Na_2S to a suspension of the mercaptide resulted in the formation of a copious, black precipitate of HgS.
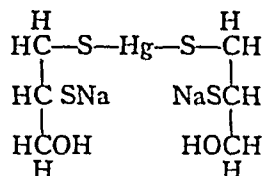
Of greater importance than the formation of the insoluble Hg-BAL complex, was the reaction which took
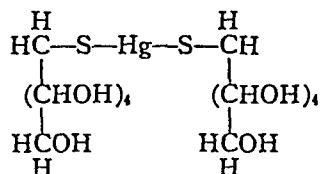
place when Hg^{++} was introduced into alkaline solutions of BAL maintained at pH 7.5. Under these conditions the addition of Hg^{++} in the molar proportion of 1 Hg : 2 BAL resulted in the formation of a complex which was soluble and so little dissociated that the addition of neutral solutions of Na_2S did not precipitate HgS. A simple formulation for the di-sodium salt of this complex (hereafter designated as $Hg(BAL)_2$) is as follows:

```
      H              H
     HC—S—Hg—S—CH
      |              |
     HC SNa       NaSCH
      |              |
     HCOH         HOCH
      H              H
```
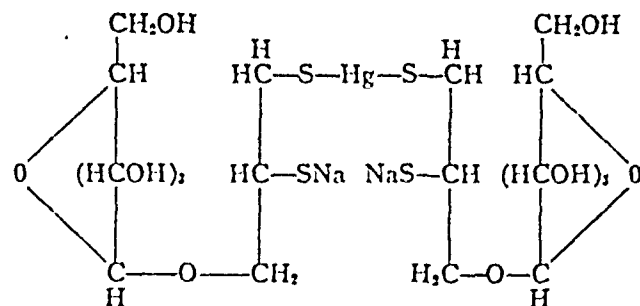
Evidence was also obtained that soluble complexes containing mercury and BAL in the molecular ratios of 2 : 3 could also be formed at slightly alkaline pH.

Reactions with 1-thiosorbitol. The reaction of 1 mol of $HgCl_2$ (0.1 M) and 2 mols of 1-thiosorbitol (0.05 M) in aqueous solution resulted in the liberation of 2 mols of H^+ as determined by titration and the formation of a complex which presumably has the following structural formula:

```
      H              H
     HC—S—Hg—S—CH
      |              |
   (CHOH)₄      (CHOH)₄
      |              |
     HCOH         HCOH
      H              H
```

The complex, hereafter designated as $Hg(thiosorbitol)_2$, dissociates to the extent that the addition of neutral solutions of Na_2S results in the copious precipitation of HgS.

Reactions with BAL glucoside. BAL glucoside[4] was obtained as the barium salt. The compound contained approximately theoretical amounts of barium and sulfur, but only 60 to 70 per cent of theoretical SH activity. The sodium salt of the glucoside was formed by the addition, to solutions of the barium salt, of a slight excess of Na_2SO_4 and the pH adjusted to *ca.* 7.4 by the addition of HCl. The $BaSO_4$ was removed by centrifugation, and aliquots of the filtrate were titrated iodometrically for SH activity. The molar equivalence of the reactions below was calculated in terms of dithiol activity as determined analytically. When solutions of 0.1 M $HgCl_2$ were reacted with those of 0.05 M BAL glucoside in the molar proportion of 1 : 1, the addition of neutral solutions of Na_2S resulted in the precipitation of HgS. Presumably a soluble mercaptide (Hg-BAL glucoside) with appreciable dissociation was formed. The further addition of a second mol of BAL glucoside in an alkaline medium (pH 7.5 or greater) resulted in the formation of a complex (Hg-(BAL glucoside)₂) unaffected by Na_2S which may be considered to have the following structure:

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[4] Received from Dr. B. W. Howk and Dr. W. H. Vinton of the du Pont Laboratories, Wilmington, Del.

```
 CH₂OH                              CH₂OH
   |        H          H              |
  CH      HC—S—Hg—S—CH           HC
  / \      |              |          / \
 O  (HCOH)₃  HC—SNa  NaS—CH  (HCOH)₃  O
  \  |                              |  /
   C——O——CH₂      H₂C—O—C
   H                        H
```

Treatment of systemic mercury poisoning in rabbits

For the determination of the efficacy of mercaptans in the treatment of acute systemic mercury poisoning in rabbits, solutions of $HgCl_2$ were administered intravenously. The lethality of 0.3 per cent solutions of $HgCl_2$ to adult rabbits of mixed sex is shown in Table I. A standard dose

The toxicity of $HgCl_2$ administered intravenously in rabbits

Dose $HgCl_2$	Mortality	Day of death				
		0 to 2	2 to 4	4 to 6	6 to 8	8 to 14
mgm. per kgm.						
2.0	6 out of 10		1	2	2	1
3.0	78 out of 81	6	39	22	6	5
5.0	2 out of 2		1	1		
10.0	1 out of 1	1				
25.0	2 out of 2	2				

of 3.0 mgm. per kgm. (LD_{90}) was employed as a background for the therapeutic study. The majority of rabbits receiving this dose died between the second and sixth days. Deaths, other than those which occurred acutely, were invariably associated with renal insufficiency as evidenced by the marked and progresive elevation of serum NPN or urea N. The determination of either NPN (micro-Kjeldahl) or urea N (Van Slyke and Cullen) was routinely performed 2, 4 and 14 days after the administration of mercury. Even after the injection of 2.0 mgm. per kgm. of $HgCl_2$, 100 per cent of animals showed marked elevations in NPN, although a small percentage survived.

The effect of treatment with BAL, 1-thiosorbitol or BAL glucoside on the course of acute mercury intoxication in rabbits is presented in Table II. If treatment was initiated within 5 minutes, 3 mercaptans were completely effective in preventing death when given in the total dose of 0.3 mM. per

TABLE II

The effect of mercaptan therapy in rabbits following the intravenous administration of an LD_{98} of $HgCl_2$

Interval before initiation of therapy	Dose of Thiol*			Mortality	Incidence of renal insufficiency in animals surviving 48 hrs.	Time of death in days		
	BAL	1-Thiosorbitol	BAL glucoside			0 to 4	4 to 8	8 to 30
min. 5	mM. per kgm.	mM. per kgm.	mM. per kgm.					
	3×0.1			0 out of 10	1 out of 10			
	3×0.05			0 out of 9	0 out of 9			
	3×0.008			3 out of 10	10 out of 10	1		2
	1×0.011			8 out of 10	9 out of 9	1	2	5
		3×0.1		0 out of 10	2 out of 10			
		3×0.05		4 out of 10	10 out of 10	2	1	1
			3×0.1	0 out of 10	0 out of 10			
			3×0.05	1 out of 10	0 out of 10	1		
			3×0.011	2 out of 10	3 out of 9	1		1
30	5×0.1			6 out of 10	8 out of 10	2	3	1
	3×0.1			2 out of 10	10 out of 10	1	1	
	3×0.05			5 out of 10	10 out of 10	2	1	2
		3×0.8		9 out of 10	6 out of 6	6	1	2
		3×0.4		2 out of 8	7 out of 7	1	.	1
		3×0.1		6 out of 10	9 out of 10		5	1
			3×0.1	4 out of 10	7 out of 9	1	2	1
			3×0.05	4 out of 9	6 out of 9		2	2
60	3×0.1			10 out of 10	10 out of 10	1	5	4
	3×0.05			8 out of 10	10 out of 10	3	2	3
		3×0.4		8 out of 10	9 out of 9	2	5	1
			3×0.1	8 out of 10	10 out of 10	1	1	6

* BAL and 1-Thiosorbitol given in 3 equally divided doses, the first, intravenously, and the second and third, intramuscularly, respectively 1 and 3 hours later. Any subsequent doses were given at 2-hour intervals. BAL glucoside given similarly, except that all injections were intravenous. BAL administered in propylene glycol (0.5 mM. per ml.). 1-Thiosorbitol and BAL glucoside administered in aqueous solution.

kgm. Of even greater significance was the fact that a rise in NPN was observed in only 3 of the 30 animals. When the dose was reduced to a total of 0.15 mM. per kgm., the superiority of the dithiols, BAL and BAL glucoside, over the monothiol, 1-thiosorbitol, became apparent. It is also of interest to observe that BAL in the total dose of only 0.025 mM. per kgm. reduced mortality to 30 per cent, although the incidence of renal insufficiency was 100 per cent.

The fact that BAL does not represent the ultimate goal in the therapy of mercury poisoning with dithiols, is shown by the superiority of BAL glucoside when compared with BAL at the dose level of 0.011 mM. per kgm. This dose was chosen because it is the molar equivalent of the dose of $HgCl_2$ employed. Thus, 5 minutes after the intravenous injection of $HgCl_2$, the administration of a molar equivalent of BAL glucoside resulted in so effective a combination of BAL glucoside with mercury as to reduce mortality to 20 per cent, and prevent renal damage in the majority of surviving animals.

The adverse effect of a delay in treatment can be seen from the data in Table II. In the rabbit, a therapeutic delay of 30 minutes resulted in an incidence of renal insufficiency of close to 100 per cent. However, all 3 mercaptans were still capable of preventing the death of a high proportion of animals. The data at 30 minutes suggest that increasing the total dose of BAL by extending the period of treatment afforded no greater protection. If treatment was delayed for 60 minutes only an occasional animal survived.

The toxicity of the preformed complexes

Information on the basic mechanism involved in the treatment of mercury poisoning by mer-

TABLE III

A comparison of the toxicities of Hg(BAL)₂, Hg(1-thiosorbitol)₂, and Hg(BAL glucoside)₂ administered intravenously in rabbits

Dose of preformed complex				Mortality	Incidence of renal insufficiency*	Day of death			
Hg(BAL)₂	Hg(1-thio-sorbitol)₂	Hg-BAL glucoside	Hg(BAL glucoside)₂			0 to 2	2 to 4	4 to 6	>6
$mgm.\ HgCl_2$ per kgm.	$mgm.\ HgCl_2$ per kgm.	$mgm.\ HgCl_2$ per kgm.	$mgm.\ HgCl_2$ per kgm.						
3.0				6 out of 6	6 out of 6		3		3
	3.0			6 out of 6	5 out of 5	1	· 5		
		3.0		1 out of 6	4 out of 6				1
		10.0		4 out of 4	3 out of 3	1		2	1
		25.0		3 out of 3	3 out of 3		2	1	
			3.0	0 out of 6	1 out of 6				
			10.0	0 out of 4	4 out of 4			1	
			25.0	3 out of 4	3 out of 3				2

* In animals surviving 48 or 72 hours.

captans was obtained from observations on the toxicity of the preformed complexes of BAL, 1-thiosorbitol and BAL glucoside with mercury. The data presented in Table III show that in terms of molar equivalent amounts, the toxicity of Hg(BAL)₂ was as great as that of HgCl₂. The same was true of Hg(thiosorbitol)₂. However, Hg-BAL glucoside was significantly less toxic than, and Hg(BAL glucoside)₂ was approximately only ⅛ as toxic as, a molar equivalent amount of HgCl₂. The paradoxical fact that a mercaptan effective in the treatment of mercury poisoning forms a complex *in vitro* which, when administered intravenously, retains the full toxicity of Hg++, will be discussed below.

Treatment of systemic mercury poisoning in dogs.

The efficacy of BAL and BAL glucoside in the treatment of systemic mercury poisoning in dogs was determined following both oral and intravenous administration of HgCl₂.

Treatment of intravenous mercury poisoning. Control studies on the intravenous toxicity of HgCl₂ in dogs revealed that a dose of 4.0 mgm. per kgm. (0.4 per cent solution) was invariably fatal. The majority of dogs died by the 4th day. All of the animals which survived 48 hours showed a marked elevation in serum urea N, the average level at this time being approximately 150 mgm. per cent. In those dogs which survived 96 hours, the serum urea N ranged between 200 and 300 mgm. per cent. One animal died within 24 hours

with evidence of massive pulmonary edema. The fact that mercury administered intravenously can damage the pulmonary capillary bed has also been observed by Rosenthal (4). The evaluation of BAL in the treatment of acute intravenous mercury poisoning in the dog is complicated by the fact that approximately 50 per cent of animals, treated within the first hour, die acutely during the first day of massive pulmonary edema (Table IV). It is known that BAL itself is capable of producing pulmonary edema as a result of a direct toxic action on the capillaries (5). Although the dose of BAL necessary to produce acute deaths from its action on the pulmonary circulation is greatly in excess of that employed in the present study, nevertheless, the possibility of a synergistic action of BAL and mercury on the pulmonary capillary bed must be considered.

Table IV depicts the efficacy of BAL and BAL glucoside when given at varying intervals of time after intravenous HgCl₂. When treatment with BAL was delayed for 30 minutes, 6 of 13 animals died acutely of massive pulmonary edema. The surviving animals enjoyed an uneventful recovery and none showed any significant elevation in serum urea N. A delay of 1 hour before the initiation of BAL therapy again resulted in the acute death of 6 of 13 animals. Of the 7 survivors, 4 ultimately succumbed, but death was significantly delayed. Three of these animals exhibited a marked rise in serum urea N, and their deaths must be attributed to renal insufficiency induced by mer-

cury. One animal survived 11 days, and at no time gave evidence of renal impairment. The 3 survivors showed no effects from the administration of mercury. Of 4 dogs treated with BAL glucoside 1 hour after the administration of mercury, none succumbed and only 1 showed a transient rise in serum urea N to a level of 70 mgm. per cent.

When treatment was delayed for 2 hours, BAL still afforded significant protection. One of 5 animals succumbed to acute pulmonary edema. Of the 4 others, only 1 died with the typical symptoms of mercury poisoning; 1 other animal showed a transient rise in serum urea N to 40 mgm. per cent. BAL glucoside was also highly effective after a therapeutic delay of 2 hours. Only 1 of 6 animals succumbed. Death occurred on the eighth day, with a characteristic rise in serum urea N concentration. Of the 5 animals which survived, none showed an elevation of urea N to levels higher than 33 mgm. per cent.

In a group of 8 dogs, treatment with BAL glucoside was delayed for 3 hours. Although 6 of the 8 animals succumbed, in 4 death was significantly delayed, occuring 8, 12, 12 and 29 days after mercury, respectively. In 2 of these animals the serum urea N rose only to 111 and 136 mgm. per cent, and was declining at the time of death. They showed progressive inanition and might have been saved with adequate supportive care.

Of the 2 surviving animals, 1 was completely protected by therapy with BAL glucoside, and the other showed transient elevation of serum urea N to a level of 42 mgm. per cent.

Treatment of oral mercury poisoning. A dose of 30 mgm. per kgm. of $HgCl_2$, given as a 1 per cent solution (LD_{100}), was employed to test the efficacy of BAL and BAL glucoside in the treatment of oral mercury poisoning. The dogs were starved for 24 hours and given 5 mgm. per kgm. of morphine sulfate, intramuscularly, 45 to 60 minutes before the administration of $HgCl_2$ to prevent vomiting. The solution of $HgCl_2$ was introduced by stomach tube. No animal which vomited was considered in the data. It should be emphasized that no lavage or local antidotal therapy was attempted, the animals being completely dependent upon the parenteral administration of the mercaptan to overcome the local effects of mercury within the gastrointestinal tract. In some experiments, both the control and experimental animals received supportive therapy 24 hours after the administration of mercury in the form of an intravenous infusion of 50 ml. per kgm. of 0.9 per cent NaCl and 5.5 per cent glucose, in order to alleviate the dehydration resulting from the severe bloody diarrhea which invariably developed. However, this inadequate procedure failed significantly to influence the course of the animals, and was not routinely employed.

TABLE IV

The effect of BAL and BAL glucoside therapy in dogs receiving 4.0 mgm. per kgm. of $HgCl_2$ intravenously ($>LD_{100}$)

Time of initiation of therapy*		Number of animals	Acute pulmonary deaths	Subsequent mortality	Incidence of renal insufficiency in animals surviving 48 hrs.**	Time of death in days		
BAL	BAL glucoside					1 to 4	4 to 7	7 to 30
hrs.	hrs.							
		23	1	22 out of 22	17 out of 17	15	6	1
½		13	6	0 out of 7	0 out of 7			
1		13	6	4 out of 7	3 out of 7		3	1
2		5	1	1 out of 4	2 out of 4		1	
	1	4	0	0 out of 4	1 out of 4			
	2	6	0	1 out of 6	1 out of 6			1
	3	8	0	6 out of 8	7 out of 8	1	1	4

* 0.15 mM. per kgm. of dithiol in 3 equally divided doses. First BAL dose given intravenously, 2nd and 3rd doses intramuscularly, 2 and 4 hours later, respectively. (Four of the dogs treated with BAL 1 hour after mercury received the first dose intramuscularly.) BAL glucoside given similarly, except that all injections were intravenous. BAL in propylene glycol (0.5 mM. per ml.) used intravenously; BAL in peanut oil (10 per cent solution) used intramuscularly. BAL glucoside administered in aqueous solution.

** Serum urea N levels greater than 35 mgm. per cent.

The effects of therapy with BAL and BAL glucoside on oral mercury poisoning are summarized in Table V. Of 19 control animals, none survived,

TABLE V

The effect of BAL and BAL glucoside therapy in dogs receiving 30.0 mgm. per kgm. of HgCl₂ orally (>LD₁₀₀)

Time of initiation of therapy*		Mortality	Incidence of renal insufficiency in animals surviving 48 hrs.**	Time of death in days			
BAL	BAL Gluco-side			0 to 2	2 to 4	4 to 7	>7
hrs.	hrs.	19 out of 19	15 out of 15	4	8	4	3
2		4 out of 10	1 out of 10			3	1
3		7 out of 15	2 out of 9	2	2	2	1
5		2 out of 5	2 out of 5		2		
	2	1 out of 8	0 out of 8			1	
	5	2 out of 6	0 out of 4	2			

* 0.15 mM. per kgm. of dithiol in 3 equally divided doses. Dogs receiving treatment 2 or 3 hours after mercury given dithiol at 2-hour intervals. Dogs receiving treatment 5 hours after mercury given 2nd and 3rd doses 2 and 17 hours later, respectively. All BAL injections were intramuscular (10 per cent solution in peanut oil). BAL glucoside administered intravenously in aqueous solution.
** Serum urea N values greater than 35 mgm. per cent.

and all those alive at 48 hours showed elevated serum urea N values, the average being 100 mgm. per cent. This increased progressively to the time of death. Only 3 animals lived for 7 or more days.

When dogs which had received oral mercury were treated 2 hours later with BAL, a high degree of protection was afforded (Table V). Six of 10 animals survived. What is more, as can be seen from Table VI, only 1 of the 10 animals showed evidence of renal insufficiency as judged by a significant rise in the level of serum urea N. In those dogs which died, death could be attributed to a severe hemorrhagic gastro-enteritis. Even more striking results were obtained when treatment with BAL glucoside was instituted 2 hours after the administration of HgCl₂. Of 8 treated animals, only 1 succumbed, and in this dog there was no evidence of a significant degree of renal impairment at 48 hours (Table VI).

When BAL was administered 3 hours after HgCl₂, 7 of 15 treated animals succumbed. Again only 2 dogs showed elevated serum urea N levels at 48 hours; 1 animal had a level of only 44 mgm. per cent and died 6 hours later. This animal was dehydrated from a bloody diarrhea. The second

TABLE VI

The effect of BAL and BAL glucoside therapy in dogs initiated 2 hours after the oral administration of 30 mgm. per kgm. of HgCl₂ (>LD₁₀₀)

Dog no.	Time of death	Serum urea nitrogen (mgm. per 100 ml. serum)				
		Before HgCl₂	48 hrs.	96 hrs.	120 hrs.	2 to 2½ wks.
After BAL therapy						
45	S*	10.5	8.8	11.9		9.0
46	17 days	5.2	5.6	7.8		
47	S	8.6	12.7	19.8		11.4
48	S	15.4	16.7	23.0		16.1
49	144 hrs.	9.4	15.4	46.3		
32	168 hrs.	17.7	10.4	9.3		
33	S	17.8	15.2	12.7		11.4
34	144 hrs.	8.9	9.6	11.8		
35	S	19.5	19.9	23.0		21.2
58	S	9.8	11.2	14.5		
After BAL glucoside therapy						
108	S	9.0	7.0		20.7	6.1
109	S	7.9	7.8		6.2	5.5
110	S	6.3	5.1		4.9	4.6
111	S	9.3	9.3		32.4	12.7
112	120 hrs.	9.7	15.5			
113	S	10.6	10.7		9.5	9.5
114	S	7.7	5.4		5.9	9.6
115	S	13.7	15.5		11.8	13.8

* S = Survival.

dog showed a progressive rise in serum urea N. In none of the 5 remaining dogs which succumbed could death be attributed to the toxic actions of mercury on the kidney.

Of the 5 animals treated with BAL 5 hours after the administration of HgCl₂, 3 survived. The 2 deaths occurred on the third and fourth days in animals with 48-hour serum urea N levels of 57 and 52 mgm. per cent, respectively. BAL glucoside was also highly effective when given 5 hours after oral poisoning with mercury. Two of 6 animals succumbed, 1 within 24 hours and the other within 48 hours; both exhibited a severe hemorrhagic enteritis. Of the 4 surviving animals, none showed evidence of systemic mercury poisoning.

DISCUSSION

It is evident from the above data that the 3 mercaptans investigated were able to protect

against the systemic effects of mercury, even when treatment was delayed. In view of the ability of these compounds to form mercaptides *in vitro,* it must be assumed that similar reactions occur *in vivo*. Moreover, the fact that early treatment with mercaptans affords complete protection, indicates that the mercaptides formed *in vivo* are sufficiently non-dissociated to prevent combination of Hg++ with essential cellular enzymes. Lastly, the observation that the dithiols are therapeutically effective when administered 2 to 3 hours after intravenous mercury in dogs, indicates that the dithiols can remove mercury already combined intracellularly.

Of the 3 mercaptans studied, the order of decreasing efficacy was BAL glucoside, BAL, and thiosorbitol. Thiosorbitol possesses certain pharmaceutical advantages over BAL in that it is a crystalline, water-soluble compound which may be readily administered by intravenous injection. Although it is much less toxic than BAL in mice and rabbits, the greater efficacy of BAL at dose levels which are well within the range of human tolerance (3) favors the choice of the dithiol for clinical use. The fact that the monothiols were less effective than the dithiols in reversing arsenic linkage with proteins has been demonstrated by British investigators (2). By analogy, monothiols might be expected to be less effective in the treatment of mercury poisoning. The *in vitro* observations reported above indicated a greater dissociation of Hg(thiosorbitol)$_2$ than of either Hg-(BAL)$_2$ or Hg(BAL glucoside)$_2$.

In dogs there was definite evidence that BAL glucoside was more effective than BAL in the treatment of both oral and intravenous mercury poisoning. This fact is chiefly of academic interest at the present time, inasmuch as BAL glucoside is not available in a sufficiently pure form to warrant clinical trial. However, it is not unlikely that compounds superior to BAL in protecting against the toxic effects of heavy metals would result from further investigations on dithiols.

Sufficient data are not available to account for the greater efficiency of the glucoside. However, certain conclusions may be drawn tentatively from observations on the toxicities of the preformed complexes. Hg(BAL)$_2$ was no less toxic than HgCl$_2$ on a molar basis. On the other hand, Hg-

BAL glucoside was definitely less toxic than inorganic mercury. Inasmuch as the available data indicate that Hg-BAL glucoside dissociates to a greater extent than does Hg(BAL)$_2$, one cannot relate the observed differences in toxicity to dissociability.

It is known that BAL is readily oxidized in the body. Therefore, the undiminished toxicity of BAL complexes could be explained by the intracellular oxidation of the complexes with the release of cationic mercury. In the treatment of mercury poisoning, a sustained concentration of BAL is maintained by repeated injections. Thus, a recombination of Hg++ released by oxidation with available BAL is possible. During the time that BAL is available, renal excretion of the complex can be effected. The fact that BAL promotes the excretion of arsenic (2, 3) and cadmium (6) has already been reported. Data on the effects of mercaptans on the excretion of other heavy metals are not yet available.

Danielli and coworkers (1) have suggested that BAL glucoside remains extracellular in its distribution. An extracellular volume distribution of the Hg-BAL glucoside complexes would explain their decreased toxicities. In keeping with this explanation, Hg(BAL glucoside)$_2$, a compound of higher molecular weight than Hg-BAL glucoside, might be expected to penetrate cells more slowly and thus be less toxic. However, it must also be assumed that the difference in the degree of dissociation of the 2 complexes may play some rôle in their relative toxicities.

An extracellular distribution of a mercaptan would not preclude the possibility of the mercaptan's removing heavy metals from intracellular enzymes. A significant degree of dissociation of the metallo-enzyme complex would result in an appreciable concentration of diffusible cationic metal which would be available for combination with the mercaptan at an extracellular site to form a mercaptide of lower dissociability. Thus, a continuous removal of metal from the cell could be accomplished.

The fact that BAL, at dose levels within the range of human tolerance, is highly effective in preventing systemic effects of mercury following oral mercury poisoning in dogs provides the experimental background for the clinical use of BAL in mercury poisoning in humans. In the above

study, none of the dogs received the adjuvant and supportive therapy which would be afforded human patients. Thus in the majority of animals, the metabolic disturbances resulting from the severe diarrhea, which was invariably present before delayed treatment was initiated, could only be corrected by voluntary ingestion of food and water, and adequate renal function. The fact that treatment was so efficacious that a majority of dogs survived even when therapy was delayed for 5 hours, attests to the value of BAL in the treatment of mercury poisoning more convincingly than if 100 per cent survived with the aid of intensive local and supportive therapy. In support of this statement is the fact that in only 1 instance did an orally poisoned, BAL-treated dog die in uremia.

SUMMARY AND CONCLUSIONS

1. Three mercaptans, BAL, BAL glucoside and 1-thiosorbitol, have been employed in the treatment of acute intravenous $HgCl_2$ poisoning in the rabbit. BAL and BAL glucoside have been employed in the treatment of acute intravenous and oral $HgCl_2$ poisoning in the dog.

2. Rabbits receiving 3.0 mgm. per kgm. of $HgCl_2$ (LD_{90}) intravenously, were completely protected by 3 doses of 0.1 mM. per kgm. of any of the mercaptans, provided therapy was initiated within 5 minutes. Therapy was progressively less effective with decreasing dosage or increasing delay before initiation. The order of decreasing efficacy of the 3 mercaptans was BAL glucoside, BAL and 1-thiosorbitol.

3. Dogs receiving 4.0 mgm. per kgm. of $HgCl_2$

intravenously (LD_{100}), were completely protected from the renal effects of the metal by 3 equal doses of BAL totaling 0.15 mM. per kgm. when treatment was delayed for 30 minutes. Striking protection was still afforded both by BAL glucoside and by BAL when treatment was delayed for 2 hours.

4. Of 44 dogs receiving 30 mgm. per kgm. of $HgCl_2$ orally (LD_{100}) in which treatment with a total of 0.15 mM. per kgm. of BAL or BAL glucoside was delayed for from 2 to 5 hours, 28 survived and only 1 animal died in uremia. No local therapy was employed, and the deaths which occurred could be attributed to gastro-enteritis and inanition.

5. The reactions between mercaptans and mercury *in vitro* have been studied, and tentative formulae for the mercaptides formed have been presented. The toxicities of the preformed mercaptides have been determined, and the mechanism of action of mercaptans in detoxifying mercury discussed in the light of these data.

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CLINICAL USES OF 2,3–DIMERCAPTOPROPANOL (BAL). XI. THE TREATMENT OF ACUTE MERCURY POISONING BY BAL [1]

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The extensive investigations upon the mechanism by which arsenic poisons the protoplasm of cells (1, 2) and the discovery (1) that the di-thiol, 2,3-dimercaptopropanol or BAL (British Anti-Lewisite), possesses an avidity for Lewisite and trivalent arsenicals, thus sparing injury to the cells and their essential enzymes, led to the suggestion that the toxic action of other metals might be explained in a similar manner. Evidence is now at hand to show that the principles involved in the injurious effect produced by mercury and cadmium are analogous to those ascribed to arsenic (1 to 3).

It has, in addition, been amply demonstrated (4) that BAL is a highly effective antidote to acute mercury poisoning in rabbits and dogs. In order to obtain complete or even partial protection against the poisonous effect of mercury bichloride, BAL had to be administered shortly after the injection of mercury; but when the first intramuscular dose of BAL was given 5 minutes after the intravenous injection of an amount of mercury bichloride fatal to the control animals, all of the rabbits survived. If, on the other hand, an interval of 30 minutes had elapsed, only about ¾ of the animals could be saved. In dogs, however, this interval could be prolonged, and when the lethal dose of mercury bichloride was given by mouth, BAL proved to be an effective antidote after the lapse of several hours. In one series of experiments, 3 of 5 dogs survived a lethal oral dose of mercury bichloride when the first intramuscular injection of BAL was made 5 hours later, followed by subsequent injections at 2 and 4 hours.

From the information available it seemed desirable to test the efficacy of BAL in the treatment of acute mercury poisoning in man. This paper, therefore, presents the observations made on 23 patients who were admitted to The Johns Hopkins Hospital with a history of having swallowed from 0.5 gram to 20 grams of mercury bichloride, and who were treated with BAL.[2]

On admission to the accident ward of Johns Hopkins Hospital, the stomach was lavaged with 5 or 10 per cent sodium formaldehyde sulfoxylate, and 300 mgm. of a 10 per cent solution of BAL in benzyl benzoate and peanut oil was injected intramuscularly. One to 2 hours after this initial dose the patient was given 150 mgm. of BAL, which was usually followed in 4 to 6 hours by another dose of 150 mgm. In several patients still a third dose of 150 mgm. was injected before 12 hours had elapsed. In this manner, 3 patients received 450 mgm. of BAL in 12 hours; 12 patients received 600 mgm; one patient, 620 mgm.; and 5 patients, 750 mgm. During the second 12 hours the patients usually received 1, or often 2, injections of BAL. Thereafter, 2 doses of 150 mgm. a day were given, as a rule, for 1 to 2 days or more, depending somewhat upon the general condition of the patient. The total amounts of BAL given 18 patients ranged from 1200 mgm. to 2870 mgm, the majority (14) receiving between 1200 mgm. and 1950 mgm.

Since many of the patients were young women weighing between 45 and 65 kgm., the first dose of BAL was somewhat larger than is usually advised (5 to 7), for it has been stated that an injection of 5 mgm. per kgm. gives rise to toxic

symptoms in about 50 per cent of normal men. In many of our patients the first injection of 300 mgm. amounted to 5 mgm. per kgm., and in a few, to 7 mgm. per kgm., and a total of the doses administered in the first 12 hours would often have been unwarranted except for the extremely serious condition of the patients. These rather excessive amounts of BAL were given with considerable trepidation, but symptoms of intoxication proved uncommon. One patient developed tingling of the tongue following the last few doses of 150 mgm., and an occasional patient complained of abdominal pain within 20 minutes after the first injection. In many patients a rise in blood pressure was recorded during the first 24 or 48 hours, but in the absence of other symptoms it is dubious whether this change can be ascribed to the injections of BAL.[3]

Immediately after admission to the hospital a series of special examinations were instituted. The vomitus, stools and urine were collected during the first few days for qualative analysis of mercury by Mr. Harry Eisenberg. Repeated chemical analyses of the blood were made by Mrs. White and her asistants. Non-protein nitrogen, chlorides, CO_2, calcium and phosphorus of the blood, and the total plasma proteins with their albumin and globulin fractions were determined repeatedly in most patients. Estimations of the phenolsulfonephthalein excretion and urea clearance were usually made on several occasions.

A summary of the essential data concerning the condition of the 23 patients is recorded in Table

I, and the results of the determinations of the electrolytes of the blood, the urea clearance and the phenolsulfonephthalein excretion are listed in Table II.

In addition to the tabulated data it was noted that all of the patients had a slight elevation of temperature during the first 24 to 48 hours after admission. The urinary output was somewhat reduced in many cases during the first 12 hours or more after admission, but the moderate oliguria did not persist in any patient, except the one who died, for more than 24 hours. At least 4 patients, including the fatal case, were admitted in shock and required transfusions of blood in addition to the usual infusions of physiological salt solution and 5 per cent glucose. A moderate elevation of blood pressure during the first 24 or 48 hours was common.

The cases have been arranged in Table I according to the amount of mercury bichloride which each patient swallowed, 8 having taken 0.5 gram or less, 6 having taken 1.0 gram, and 9 having swallowed from 1.5 grams to 20 grams. This dose was determined from information gained from several sources, but the circumstances under which some of these patients swallowed the tablets, or drank the fluid containing powder, often rendered it difficult to be certain of the exact amount swallowed.

A somewhat more detailed analysis of these groups shows, as might be expected, that the symptoms at onset, as well as the findings on admission and the clinical course during the first few days of observation, varied considerably from group to group.

The first group of 8 patients swallowed only 1 tablet, or 0.5 gram of mercury bichloride. This dose is said to be rarely, if ever, fatal. None of these patients appeared seriously sick. None showed blood in the stool and only 1 showed blood in the vomitus. Four of 5 cases in which the stools were examined gave positive tests for mercury, but mercury was not found in the vomitus of 3, or in the urine of any of the 5. Small amounts of albumin were found in the urine of 6; none in the urine of 2. None were in shock on admission, and hemoconcentration was not noticeable, although 1 patient (No. 6) had a hematocrit reading of 51. Only 2 patients showed a leukocyte count of over 15,000 per cu. cm. The

[3] Since the completion of this paper, 3 patients have shown rather pronounced reactions after 1 or more doses of BAL. One woman weighing 58 kgm. who had swallowed 1.5 grams of bichloride of mercury, experienced flushing of the face, fullness in the head, dizziness, sweating, shooting pains in arms and legs, burning of mouth and throat, and pain in epigastrium after the first dose of 300 mgm. of BAL, which amounted to 5.1 mgm. per kgm.; subsequent doses of 150 mgm., or 2.5 mgm. per kgm., gave no untoward symptoms. A second woman weighing 44 kgm. who had swallowed 0.5 gram of bichloride of mercury suffered from flushing of the face and abdominal pain after the first dose of 300 mgm. of BAL, which represented 6.8 mgm. per kgm. The second and third doses of 150 mgm. or 3.4 mgm. per kgm., produced no symptoms. The fourth dose of 150 mgm. was followed by flushing and cardiac irregularity due to extra systoles. A third patient was observed to have extra systoles following a single dose of 150 mgm. of BAL given late in the course of treatment.

TABLE I

A summary of the essential data concerning the condition of 23 patients

No.	Date	Age	Sex	Col.	HgCl₂	Seen hrs. later	Gastric lavage Na form. sulfox.	Mercury Vomitus	Mercury Stools	Mercury Urine	Leucocytes per cu.mm	Albuminuria Amt.	Albuminuria Duration days	Hematocrit	Plasma proteins grams per 100 ml.	NPN mgm. per 100 ml.	BAL 1st 12 hrs. mgm.	BAL Total mgm.	Recovery
1	11/15/45	20	F	W	grams 0.5	3½ to 4	Yes	Specimen lost			12,700	++	1	44	8.50	35	750	1350	4 days
2	9/25/45	42	M	W	0.5	1½	Yes	0	+++	0	15,520	+	2	no exam.	no exam.	no exam.	450	600	Transferred 2nd day to asylum
3	10/31/45	43	F	W	0.5	1 to 20 min.	Yes	0	0	0	10,920	+	1	43	7.38	38	450	600	Left hospital 2nd day
4	8/5/45	32	F	W	0.5	1½	Yes	no exam.	+++	0	13,650	±	3	38	no exam.	35	600	1650	4 days
5	8/14/45	26	M	W	0.5	1	Yes	0	+	0	9,300	0+±	3	48	no exam.	33	600	1350	7 days
6	11/10/45	31	M	W	0.5	20 min.	Yes	Specimen lost			10,900	+	1	51	7.75	33	750	1650	2 days
7	12/31/45	22	F	W	0.5	3½	Yes	Specimen lost			15,200	0		45	7.15 1/4/46	44	600	900	3 days
8	12/27/45	31	M	W	3 ml. paste ammoniated mercury	4½	Yes	Not done	+++	0	9,575	0		45.8	6.94	40	600	900	3 days
9	11/21/45	60	M	W	1.0	3½	Yes	++	++++	±	18,000	++	2	54	7.0	30	600	1500	3 days
10	10/21/45	25	M	W	1.0	1½	Yes	0	lost	0	15,135	0		43	7.38	38	600	1350	2 days
11	10/18/45	29	M	W	1.0	1	Yes	++++	0	0	13,380	+	1	51	8.56	35	450	1950	4 days
12	3/21/45	35	F	W	1.0	1½	Yes	+++	no exam.		12,120	0 +++	8	50	7.0	30	150	300	8+
13	6/4/45	27	F	W	1.0	2	Yes	++	no exam.		11,400	++++	2	no exam.	5.88	24	600	1200	3 days
14	9/22/44	67	F	W	1.0	13	No, In shock	no examination			21,480	+++++	9	65	no exam.	60 246	225	1575	Death 9th day
15	11/1/45	19	F	W	1.5	1½	Yes	+++	+	0	20,520	++ ±	3	47	9.19	30	600	1200	4 days
16	5/17/45	18	F	W	1.5	1½	Yes	no exam.	++++	+++	12,120	+++ ±	5	44	no exam.	31	600	1950	7 days
17	7/24/45	31	F	W	1.0± in water 20	3	Yes	0	+	0	18,100	+	1	no exam.	no exam.	47	600	1500	2½ days
18	9/16/45	31	F	W	0.5 1.5	1½	Yes	no exam.	+	++	15,000	+±	6	41	no exam.	35	600	1800	7 days
19	7/6/45	16	F	C	1.5+	19	Yes	±	+	+	28,000	12 grams ±	21	no exam.	8.69	59	620	2870	22 days
20	8/17/45	16	F	W	Powder in water	1 hr. 40 min.	Yes	++	++++	+++	12,600	+±	3	51	7.75	21	600	2400	7 days
21	10/10/45	21	F	W	In water 20	3½	Yes	0	+	0	22,000	+±	6	57.5	10.19	36	750	1650	6 days
22	12/29/45	?	F	W	3.0	2	Yes	+++ +++	+++	0	17,000	+++ +	6 ft. tr.	46.8	8.0	42	750	1350	5 days
23	1/2/46	27	M	W	2.5	1½	Yes	?	+++	0	23,000	2.8 grams ++++	2	52.3	8.63	42	750	1200	3 days

TABLE II

The results of determinations made on 23 patients.

No.	HgCl₂	Chlorides		CO₂		Urea clearance	Phthalein 2 hr. excretion	Calcium	Phosphorus
		Admission	Lowest	Admission	Lowest				
	grams	m.eq.	m.eq.	m.eq.	m.eq.		per cent	mgm. per 100 ml.	mgm. per 100 ml.
1	0.5	106.2		23.3		130	63	9.8	4.1
3	0.5	98.6		25		not done	not done	10.7	3.9
4	0.5	101		19.1		140 and 114	84	9.8	1.8
5	0.5	102	97	15.8		111	101	10.4	3.6
6	0.5	107	93.6	25.8		160	not done	10.2	2.5
7	0.5	98.4		25		not done	not done	not done	not done
8	0.1±	95.1	91	32		107 and 76	85	9.9	3.5
9	1.0	93.6		22.8		106 and 92	80	10.0	4.3
10	1.0	99	95.6	21.6		78 and 75	82	10.3	3.2
11	1.0	103		19.1		91 and 85	63 to 99	11.2	4.7
12	1.0	102.5	91.5	18.2		100	30	9.2	2.7
13	1.0	105		23.3		not done	42.5	not done	not done
14	1.0	89		21.6	11.5	not done	not done	7.9	6.2
15	1.5	103.4		19.9		60 and 54	88	10.0	4.4
16	1.5	110	99	21.2		76 and 64	90	9.5	3.8
17	1.0±	91		17.4		98	95	10.0	3.2
18	{0.5 1.5	105		14.1		115	68 to 73	9.6	3.5
19	1.5+	89		20.8	19.9	22 and 25 to 61.64	8 to 52	10.3	3.6
20	{powder in water	105	96	19.9		95	90	10.2	2.8
21	20	82		19.1		79 and 65	71	11.0	3.2
22	3	86.5		19.1		150 and 82 max.	65	not done	not done
23	2.5	101.2	95.2	20.1		81 to 88	95	11.2	4.0

The number of cases correspond to those in Table I.

TABLE III

H.B.   w.f.   age 32   No. 359135   Adm. August 5, 1945, 11:45 p.m.

*August	5	6	7	8	9
Blood pressure		110/80 130/90	130/85		
Leukocytes cu. mm.	8,000	13,650		7,400	
Hematocrit	38	36.6		38.4	
Fluids, ml.	1,500	5,730	4,750	5,120	1,120
Urine, ml.	110	4,950	3,750	5,590	525
Albumin	±	+ ±	±0	0	0
Red blood cells	0	0	0	0	0
Casts	0	± ±	±	±	0
Mercury		0 0		0	
Vomit {No.	2	0	0	0	0
blood	+				
mercury					
Stools {No.	1	1	0	0	1
blood	0				0
mercury		+++			±
Phthalein 2 hr. excretion		84 per cent		92 per cent	
N.P.N., mgm. per 100 ml.	35	26	23	30	
CO₂ m.eq.	19.1	23.3	25.8	26.6	
Chlorides m.eq.	101.0	102.0	108.0	104.0	
Urea clearance		140 to 114		160	
BAL mgm.	450	600	300	150	150
BAL total					1,650

Case 4, Table I.   Wt. 59 kgm.   One and three-quarter hours before admission to Johns Hopkins Hospital she had swallowed 1 tablet of bichloride of mercury (0.5 gram).   She vomited the tablet, swallowed it again and then drank a cup of coffee.   There was severe epigastric burning.   She was taken to Baltimore City Hospital a little later, where the stomach was lavaged with sodium formaldehyde sulfoxylate.   She was then transferred to Johns Hopkins Hospital.
   * All 24-hour determinations in these tables are calculated from midnight to midnight.

non-protein nitrogen of the blood in one was 40 mgm. per cent; in another, 44 mgm. per cent. But in the other 5 patients in which this determination was made, the figures were within normal limits. Recovery was rapid. Table III shows the course of 1 of these patients.

The second, or intermediate group, comprises 6 patients who swallowed 2 tablets, or 1.0 gram of mercury bichloride. The symptoms on admission were much more serious than in the first group. Diarrhea and persistent vomiting were common. Blood was present in the vomitus of 2 and in the stools of 2. In 1 of these the diarrhea was grossly bloody. Mercury was found in large quantities in the stools of 1 of 2 patients, and in the urine of 1 of 3 patients. One patient was admitted in collapse (No. 12), 1 in profound shock (No. 14) 13 hours after having taken 2 tablets of bichloride and having slashed her wrists. This patient, the first, treated with inadequate amounts of BAL, died 9 days later. There was evidence of hemoconcentration in 4 patients, the hematocrit being 50 or above in all of these. The leukocyte counts ranged from 15,000 to over 24,000 per cu. cm. in 3, and there was well marked albuminuria in all but 1. Except in the fatal case, recovery took place rapidly in all but No. 12, who was the second case treated, and who received inadequate doses of BAL, a total of only 300 mgm. Table IV records the detailed study of 1 patient in this group, and Figure 1 depicts the course of the fatal case.

The third group of 9 patients was admitted to the hospital in a condition that was considered to be serious, and in some, actually critical. Five had swallowed at least 3 tablets (1.5 grams) of bichloride; 1, 5 tablets (2.5 grams), 1, 6 tablets (3.0 grams); 1, an unknown quantity of powder in water; and one, at least 20 grams in water. One patient was not admitted to the hospital until 19 hours after taking 3 tablets (1.5 grams) of bichloride. In the meantime she had only been treated with eggs and milk. The remaining 7 patients were admitted from 1¼ to 3½ hours after

TABLE IV

J.C.McC. w.m. age 60 No. 269218 Adm. Nov. 21, 1945, 7:55 p.m.

November	21	22	23	24	25	26	Followup 12/17/45
Blood pressure	120/86	160/90 130/90	150/90	150/90	150/90	150/90	140/90
Leukocytes, cu. mm.	18,000	17,000	10,150	10,050		10,850	
Hematocrit	54	57	42	42		43.3	
Fluids, ml.	2,000	6,525	4,990	3,180	3,850	3,690	
Urine, ml.	100	3,605	2,790	1,250	1,325	965	
Albumin	+	+ 0	0 0	0	0	0	0
Red blood cells	+	± ±	± 0 0	0	0	0	0
Casts	+	+ +	0 0 0	0	0	0	?
Mercury	±	0 0	0				
Vomit {No.	lavage	1	0	0	0	0	
blood	++	+					
mercury}	++	+					
Stools {No.	2	7	0	0	1	0	Asymptomatic
blood	++ ++	Gross			0		
mercury}	+++ +++++	++++ 0					
Phthalein 2 hr. excretion		80 per cent					85 per cent
N.P.N. mgm. per 100 ml.	30	24	25	25		32	
CO₂ m.eq.	22.8	25.8	27.9	30		29.2	
Chlorides m.eq.	93.6	93.0	98.2	98.2		94.2	
Plasma prot., grams per 100 ml.	7.00						
Albumin, grams per 100 ml.	4.69						
Globulin, grams per 100 ml.	2.31						
Urea clearance			106 to 92				
BAL mgm.	450	450	300	300			
BAL total					1,500		

Case 9, Table I. Admitted to Johns Hopkins Hospital 3½ hours after swallowing 2 tablets of bichloride of mercury (1.0 grams). Twenty minutes after taking the tablets he vomited once. On admission the stomach was lavaged with 4000 ml. of 5 per cent sodium formaldehyde sulfoxylate. Within ½ hour he had abdominal cramps and tenesmus with bloody, watery diarrhea.
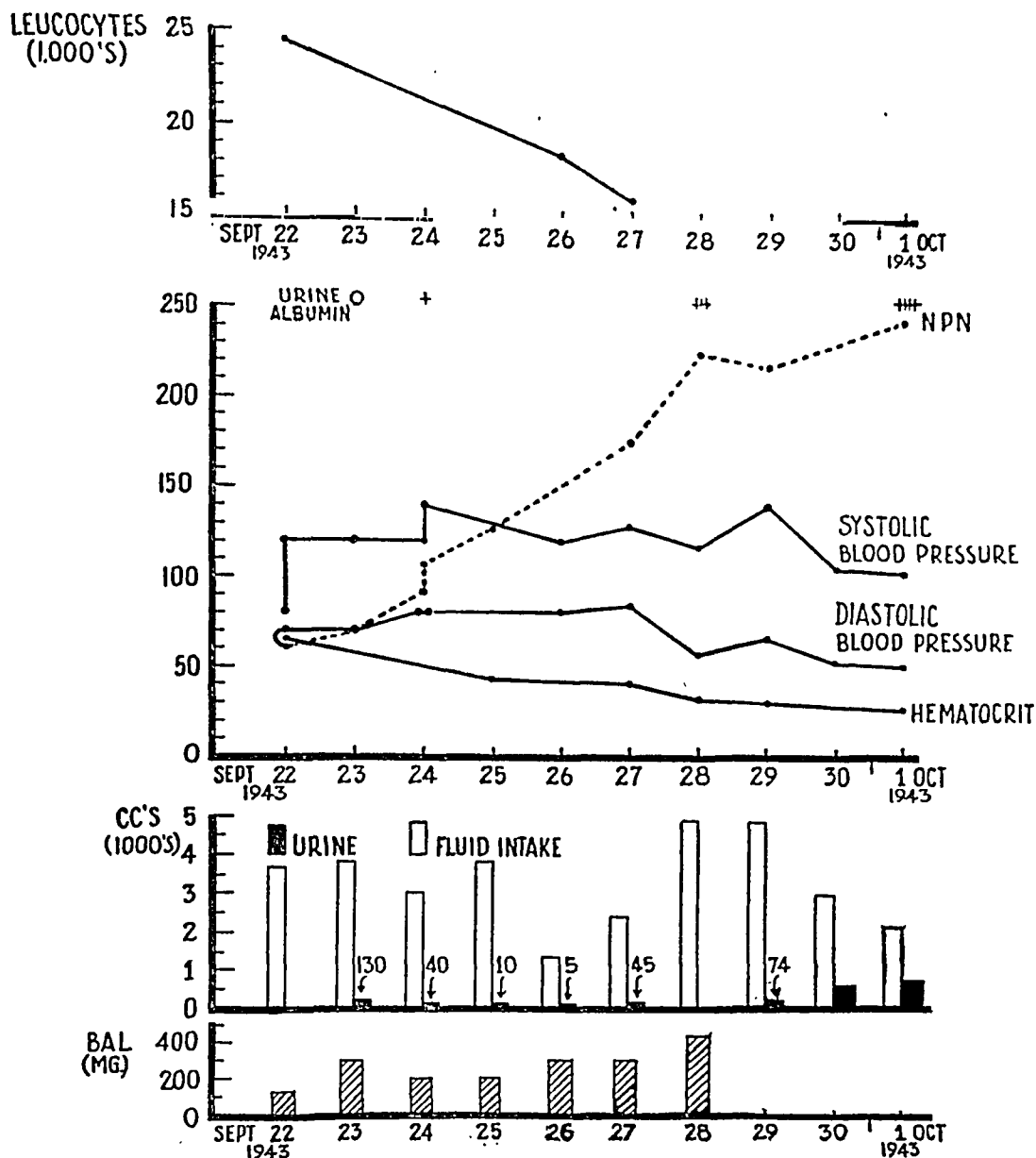
FIG. 1. CASE 14, TABLE I

Female, white, age 67, No. 224094, admitted to Johns Hopkins Hospital Sept. 22, 1943. Her right kidney had been removed in 1933 and she had had palpitation and shortness of breath for 1 year. Thirteen hours before admission at 7 a.m. she had taken 2 tablets (1.0 gram) of bichloride of mercury and had slashed her wrists. The stomach was lavaged with sodium formaldehyde sulfoxylate 8 hours after taking bichloride. She had vomited small amounts of bloody material. She had voided only once. On admission she was unconscious and in shock, the pulse unobtainable, the blood pressure 80/60, respirations 36 and temperature 101.4°. Lavage of the stomach was again performed; she was given 1000 ml. of plasma intravenously, and digitalis. She was anuric. Within 48 hours the condition of the circulation had improved and the blood pressure had risen to 140/80.

swallowing the bichloride, and had received lavage with 5 per cent sodium formaldehyde sulfoxylate either before admission or on admission to the hospital. Most of them showed hemoconcentration, the plasma proteins in 1 being 9.19 grams per 100 ml; and in another, 10.19 grams per 100 ml. At least 3 were in shock and required transfusions of blood as well as intravenous infusions of saline and 5 per cent glucose. The vomitus was bloody in 6; the diarrheal stools, bloody in 5.

The leukocytes varied from 15,000 to 28,000 per cu. cm. in 7 patients and were above 20,000 in 4 of these. All showed considerable amounts of albumin, casts and red blood cells in the urine on admission, and the non-protein nitrogen of the blood was somewhat elevated in 3 and increased to 59 mgm. per cent in 1 other. It was in this group also that the blood chlorides were most notice-
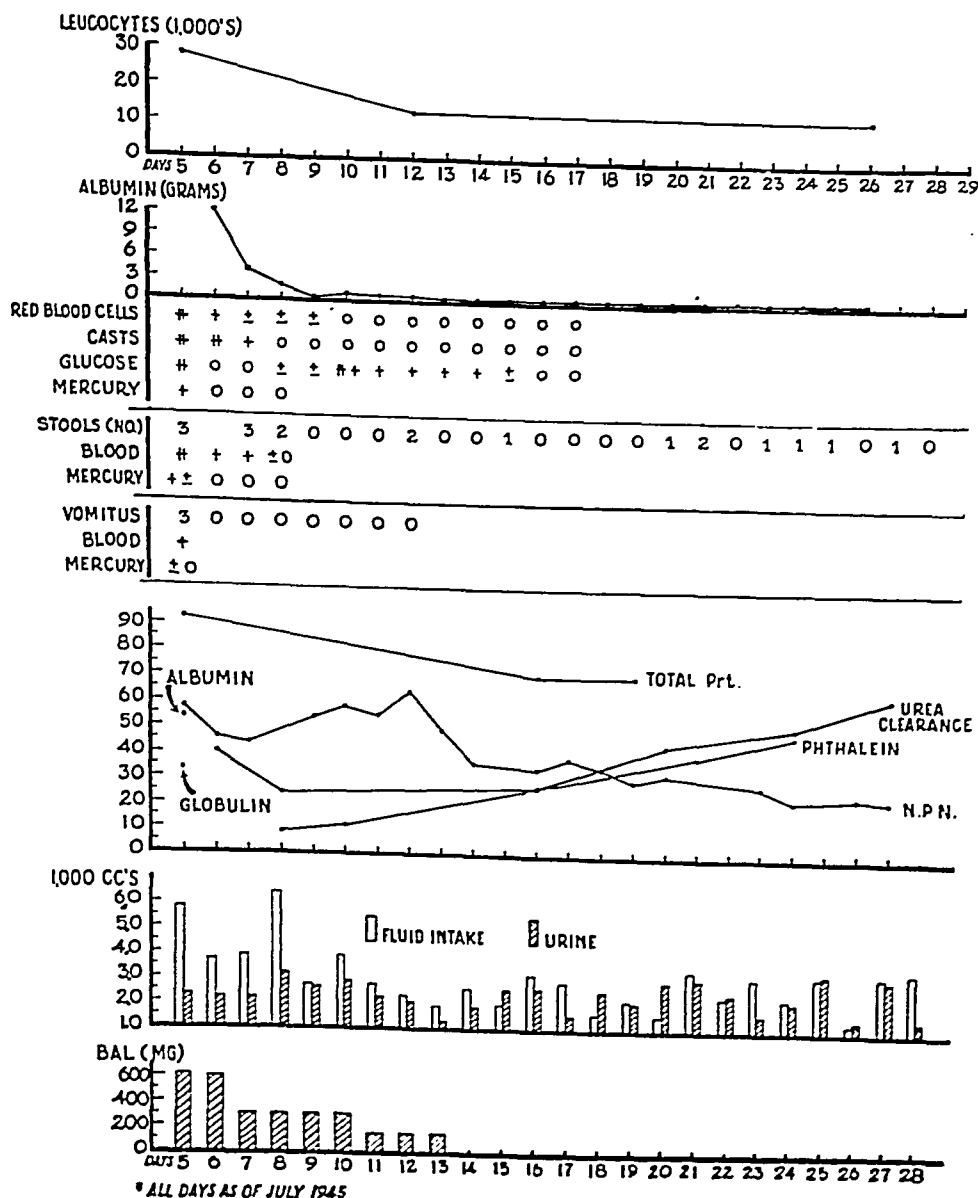

FIG. 2. CASE 9, TABLE I

Female, colored, age 16, admitted to Johns Hopkins Hospital July 6, 1945, No. 356389, wt. 120 lbs. At 10 p.m. on July 4, 19 hours before admission, she had taken 3 tablets (1.5 grams) of bichloride of mercury in water. Fifteen minutes later she drank 3 quarts of milk and vomited. Milk and eggs were administered after 1 to 2 hours. Vomiting continued and was bloody by 4 a.m. July 5. Abdominal pain continued and she became drowsy. On admission the stomach was lavaged with 5 per cent sodium formaldehyde sulfoxylate, and physiological salt solution and 5 per cent glucose administered intravenously. She passed 2 bloody stools. She was stuporous and showed pitting edema.

ably reduced, the lowest figure being 82 m. eq. This was also true of the blood bicarbonate, the lowest figure being 14.1 m. eq.

The sudden and favorable change that took place in the condition of these patients in 48 hours was often unexpected. All of them except 1 (No. 19), were symptomatically well within 2 to 3 days, and 8 had recovered entirely with 2½ to 7 days. Complete restitution to normal did not occur in No. 19, who was admitted to the hospital 19 hours after taking 3 tablets of bichloride of mercury, until 22 days, though she was free of all symptoms at the end of 2 weeks.

Figure 2 presents in detail the course of the illness in this patient; and Table V, the course in 1 other patient in this group.

The evidence that we have been able to collect through the study of these 23 patients supports the contention that BAL is capable of neutralizing the toxic action of unusually large doses of mercury bichloride. The effects are most striking when BAL is administered intramuscularly in comparatively large amounts within 3½ hours after the ingestion of mercury bichloride. Under these circumstances the kidney appears to be spared serious or lasting injury, even when mercury can be detected in the urine for many hours after the ingestion of the mercury bichloride. Our observations in man, therefore, are in accord with the experimental results reported by Gilman and co-workers (4).

The outcome in any case of poisoning by bichloride of mercury is conditioned by many factors, some of which are quite beyond control; and it is therefore very difficult to estimate the value of one form of treatment, or a combination of methods, in a series of cases as small as this. The recognition (8) of the loss of electrolytes and the dan-

TABLE V

M.F. w.f. age 24 No. 256736. Adm. Oct. 10, 1945, 10:40 p.m.

October	10	11	12	13	14	15	16
Blood pressure	80/50 92/50	62/44 106/64	96/58 114/50	96/62 120/80	92/60 116/70	92/68 100/64	96/58
Leukocytes cu. mm.	22,600			8,500		5,600	5,600
Hematocrit	57.5 200 cc. blood	46		41		46	
Fluids, ml.	1,500	7,450	1,945	1,550	3,380	1,975	760
Urine, ml.	?	3,507	1,870	2,160	2,375	2,665	?
Albumin	+	± 0	± ±	± ±	± ±	± 0	0
Red Blood Cells	±	0	0	0	0	0	0
Casts	+	+	0	0	0	0	0
Mercury	+++	0 +	0	0	0	0	0
Vomit { No.	3	4					
Vomit { blood	±	± 0					
Vomit { mercury	++++ 0	+ 0					
Stools { No.	3	3	0	1	0	0	0
Stools { blood	0	0		+			
Stools { mercury	++ + +	+ +++		0			
Phthalein 2 hr. excretion		71 per cent		45 per cent		71 per cent	
N.P.N., mgm. per 100 ml.	36	28	18	32		29	32
CO₂ m.eq.	19.1	19.9	25.8			25.8	
Chlorides m.eq.	82.0	103.0	100.8			94.8	
Total plasma prot., grams per 100 ml.	10.19			7.5			
Albumin, grams per 100 ml.	6.31						
Globulin, grams per 100 ml.	3.88						
Urea clearance			79.65			72.78	
BAL mgm.	300	900	300	0	150		
BAL total						1,650	

Case 21, Table I. Wt. 94¾ lbs. Admitted to Johns Hopkins Hospital 3½ hours after drinking warm water containing about 20 grams of bichloride of mercury. She had emptied a box containing 30 grams of bichloride of mercury into a glass of water, stirred and drank all but the dregs. She then had abdominal cramps and nausea. Later she drank milk and eggs and started vomiting about 40 minutes after drinking bichloride of mercury. On admission to the hospital the stomach was lavaged with 4000 ml. of 5 per cent sodium formaldehyde sulfoxylate. She was in shock and required 2 transfusions of blood.

ger of shock in the early stages, together with the introduction of the use of intravenous infusions of physiological salt solution and glucose, reinforced by transfusions, when necessary, marked a distinct advance in therapy which has been employed in our patients. The introduction (9) of gastric lavage with solutions of sodium formaldehyde sulfoxylate marked a still further step in advance, and though the value of this antidote has been questioned, we have availed ourselves also of its aid.

It is, however, very difficult to determine from the published data that any of these methods have, in the hands of many observers, resulted in a significant reduction in mortality when amounts of mercury bichloride greater than 1.5 grams were swallowed (9b to 11).

It has been generally stated (12) that a dose of 0.5 gram of mercury bichloride by mouth is rarely, if ever fatal; that when 1.0 gram is swallowed and vomiting does not occur within 10 minutes, the prognosis is poor (13), and that 1.5 grams often results in death.

In 263 cases of bichloride of mercury poisoning admitted to Johns Hopkins Hospital from 1925 to 1945,[4] there have been 34 deaths (12.9 per cent). One fatality (4.4 per cent) occurred in the 23 cases treated with BAL and described in this report. While this manuscript was being prepared, 2 additional patients were treated with BAL and made a rapid recovery. Including these cases, there has been 1 fatality in 25 cases (4.0 per cent). Because the severity of poisoning influences the fatality rate so greatly, we have divided the patients into groups according to dosage of poison, proteinuria, and leukocytosis on the day of admission. These features were chosen because they could be quickly determined, and appeared to have

some prognostic value (11, 14 to 17). In the control series, increasing dose, leukocytosis, and proteinuria are associated with increasing fatality rates. The severity of poisoning in the group receiving BAL, as indicated by the distribution of cases in Figure 3, appears somewhat greater than in the control series. The number of cases is too small for satisfactory statistical treatment, and only a preliminary impression of the value of BAL can be given.

It can be said, however, that the 23 cases presented in this study include patients representing many different examples of poisoning by mercury bichloride, ranging from the mildest types to those which appeared to be extremely serious, both on account of the amount of bichloride of mercury swallowed and the intensity of symptoms on admission. Perhaps the most significant effect of the treatment was the prompt relief of even the most alarming symptoms when BAL in sufficient doses was administered within 3 to 4 hours after mercury bichloride had been swallowed, and the rapidity with which the patients made a complete recovery.[5]
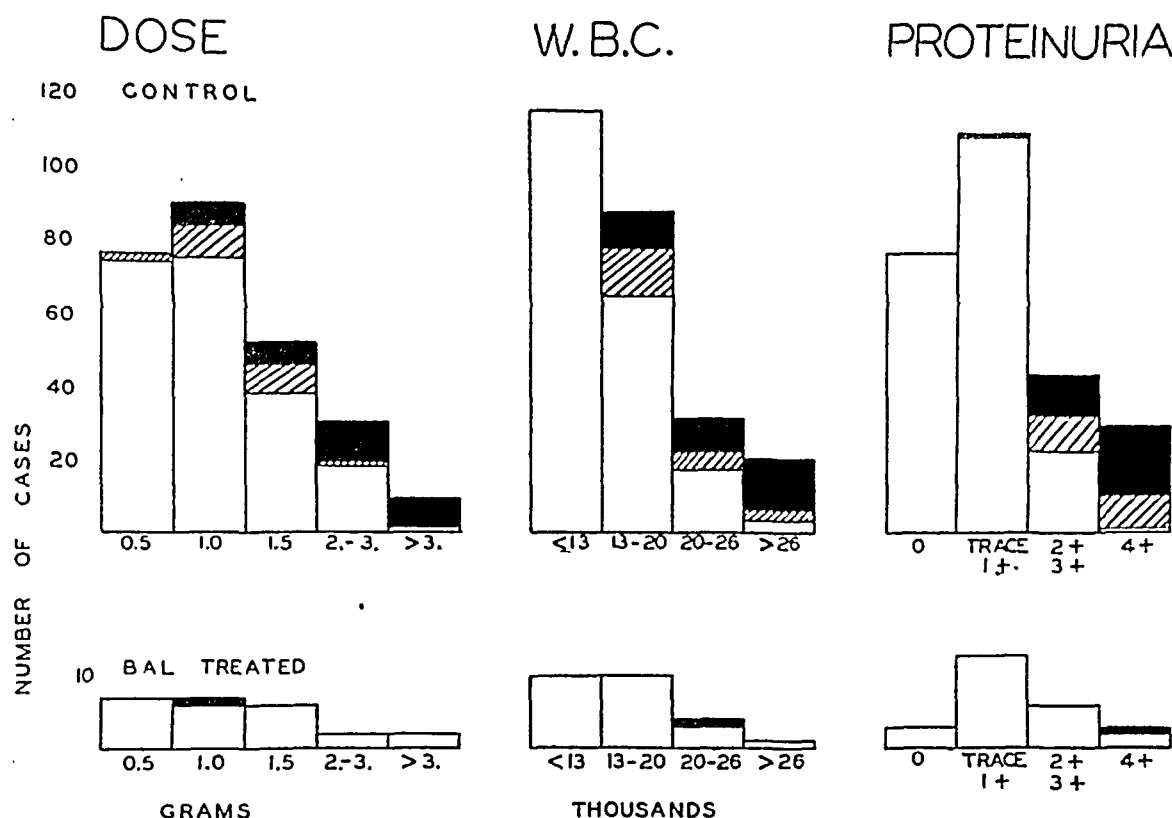
FIG. 3. DISTRIBUTION OF CASES OF POISONING BY BICHLORIDE OF MERCURY ACCORDING TO DOSAGE, LEUKOCYTOSIS, AND PROTEINURIA

The height of each column represents the number of cases with the particular dose, white blood count, or urinary protein noted below. Fatal cases are indicated in black, persistent renal damage (proteinuria or reduced renal function on subsequent examinations) in shaded areas, and recovery in white. The upper chart represents 263 cases not treated with BAL. The lower chart represents 25 cases treated with BAL. In the control series, the increasing fatality rates with increasing dose, leukocytosis, and proteinuria, are evident. The BAL series shows evidence of a probably greater severity of poisoning, as judged by these prognostic signs.

SUMMARY

Twenty-three cases of acute poisoning by mercury bichloride have been treated with intramuscular injections of BAL. Eight of these patients swallowed not more than 0.5 gram of mercury bichloride, and treatment with BAL was started from 20 minutes to 3½ hours later. All made a prompt recovery.

Six patients swallowed 1.0 gram. Five were treated within 1 to 3½ hours, all recovered within 2 to 8 days. One patient who was treated initially with small amounts of BAL 13 hours after taking 1.0 gram of mercury bichloride died on the ninth hospital day.

Nine patients took from 1.5 to 20 grams of mercury bichloride, 5 of the 9 having swallowed more than 1.5 grams. Eight patients were treated with BAL from 1¼ to 3½ hours after taking the mercury, 1 patient was first treated 19 hours after having swallowed at least 1.5 grams. This patient was entirely well in 3 weeks, and the other 8 patients recovered completely in 2½ to 7 days.

The initial amount of BAL used for the first intramuscular injection in 21 patients was 300 mgm. (3 ml. of a 10 per cent solution). Two patients, including the 1 who died, received an initial dose of only 150 mgm. Twenty-one patients received from 450 to 750 mgm. in the first 12 hours,

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these 27 died. There were 25 patients in an analogous group treated by BAL with no deaths.

Comparison of fatalities in a control group of patients who swallowed 1.0 gram or more of mercury bichloride and were admitted to the Johns Hopkins Hospital within 4 hours and an analogous group treated with BAL:

	No.	Deaths
Control Cases	86	27
Patients Treated with BAL	24	0

and a total of 900 mgm. to 2870 mgm. in a period of 3 to 4 days.

Toxic reactions attributable to BAL were observed in a few patients after the intramuscular injections of 300 mgm., or in some instances, 150 mgm., of BAL.

Considerable importance is attached to the prompt treatment by BAL in an initial intramuscular injection of 300 mgm., followed within the first 12 hours by 2 or even 3 further injections of 150 mgm. each.

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KIDNEY FUNCTION AND CIRCULATORY COLLAPSE. POST–SYNCOPAL OLIGURIA [1]

By CLAUS BRUN, E. O. E. KNUDSEN AND FLEMMING RAASCHOU

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In the course of investigations on renal function in the passive erect posture maintained on a tilt-table, we observed a number of unintentional cases of circulatory collapse. We noted that syncope was followed immediately by a reduction in urine flow which, in relation to the quantity of liquid ingested and the foregoing diuresis, was very pronounced. We have called this phenomenon "post-syncopal oliguria."

The literature reveals that similar forms of oliguria have been observed before, but without having attracted particular attention. Chasis, Ranges, Goldring and Smith (1) induced orthostatic hypotension by the ingestion of sodium nitrite, and they observed protracted oliguria which continued even after the subject had been placed in the horizontal position. Marked and abrupt reduction in urine flow in experiments utilizing the tilt-table are also recorded by Smith (2, 3). In neither case, however, is the mechanism of the oliguria discussed.

PASSIVE ERECT POSTURE

Our experiments have been concerned with the passive erect posture, in which the hydrostatic changes in the circulation which normally occur in the erect posture are especially accentuated. We have obtained this passive erect posture by placing the individual on a tilt-table, where he is supported by sitting on the saddle so that the lower extremities hang down motionless and without support. By this means the muscle pump of the lower extremities is removed from action. Passive erect posture where the tilting board forms an angle of 60° with the horizontal, and with the head upwards, is designated as + 60°.

CIRCULATORY CHANGES IN THE PASSIVE ERECT POSTURE

As a consequence of accumulation of blood in the lower parts of the body, the hydrostatic pressure in the peripheral veins below the heart increases, and with it the total cross-section of the venous system. Capillary pressure rises and blood accumulates in the tissues as edema. Central venous pressure is reduced, resulting in a decreased filling of the heart and decreased stroke volume. Through excitation of the pressor receptors the pulse is accelerated, and vasoconstriction occurs in certain parts of the body. The systolic blood pressure usually remains fairly constant, whereas the diastolic blood pressure rises as a consequence of arterial constriction, so that the pulse pressure is decreased. Our observations on blood pressure changes are in agreement with those illustrated by Smith (2, 3). This posture is tolerated by normal subjects for periods varying from minutes to about 2 hours, depending on as yet undefined physiological conditions. Sooner or later the blood pressure begins to fall, and in the course of a few minutes may drop to unmeasureably low values. This is accompanied by the common subjective and objective symptoms of syncope and terminates in the loss of consciousness, which is quickly restored when the individual is brought into the horizontal posture.

EXPERIMENTAL PROCEDURES

The individuals studied here were healthy undergraduates who received 100 ml. of water every 10 minutes throughout the experiment. A few of our experiments were carried out without water.

Glomerular filtration was measured by the inulin clearance, and renal plasma flow by the diodrast clearance. Hemoconcentration was followed by determination of the hemoglobin concentration, the cell volume and the viscosity of the blood. Occasional determinations of plasma protein concentration were also made.

The water-loaded person (or in some cases subjects without a water load) was placed on the tilt-table in the passive erect posture. Blood pressure was observed every ½ minute. When the intended degree of circulatory collapse was reached the subject was returned to the horizontal position. Normal circulation was reestablished immediately, as judged from pulse rate and blood pressure, and subjective feelings disappeared at once. Immediately after recovery the subject rose from the table and voided, which is important as it was necessary to separate the urine produced during the collapse period from that produced after syncope. The renal clearance of the brief collapse period is therefore included in the last clearance period in the passive erect posture. In the majority of experiments we employed a catheter à demeure with bladder lavage.

EXPERIMENTAL RESULTS

In all, we have made 16 series of tilting experiments including circulatory collapse, and all 16 experiments have given concordant results.

In our first experiment we observed that after a brief fall in blood pressure, there occurred a period of protracted, apparently complete, anuria. As this observation was unexpected, the subject was catheterized 40 minutes after syncope, and it was found that the bladder was completely empty, although throughout the experiment he had drunk 200 ml. of water every 10 minutes.

Figure 1 shows the diuresis curve from a typical experiment with post-syncopal oliguria. Immediately after syncope the urine flow fell to relatively low values, where it remained for about 65 minutes, thereafter rising again to the initial value. Post-syncopal oliguria lasts from 15 to about 90 minutes after syncope.

In most of our experiments the values of the blood pressure during syncope could not be observed owing to the velocity with which the blood pressure fell. Notwithstanding the inadequacy of the data, there seems to be a rough proportionality between the extent of blood pressure reduction and the duration of the period of oliguria. This supposition is supported by the following experiment. After ingesting the usual amount of water, a subject was tilted 4 times, each time with an interval in the horizontal position. The tilting angle

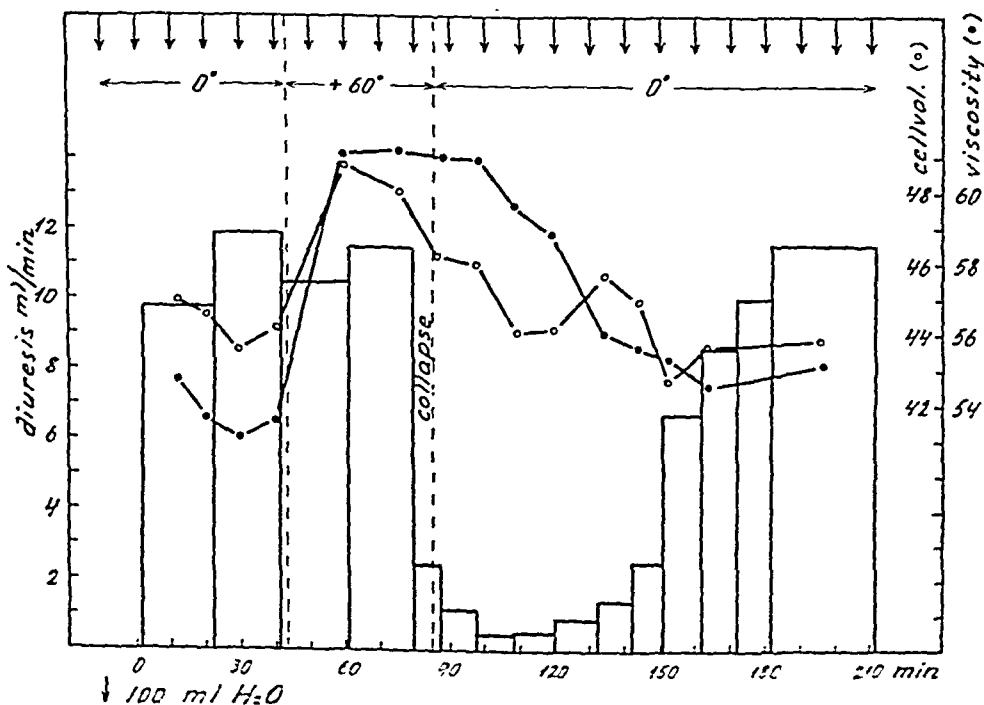FIG. 1. EXPERIMENT SHOWING OLIGURIA AND HEMOCONCENTRATION AFTER A BRIEF SYNCOPE

0°: The individual in the horizontal posture. +60°: The individual in the passive, erect posture, head upwards; the tilting board forms an angle of 60° with the horizontal plane. Columns: Urine flow (ml. per min.). Open circles: Blood cell volume (per cent); closed circles: Viscosity of the blood (seconds); arrows: Water, 100 ml. every 10 mins.

and the duration of tilting were gradually increased, by which means it was possible to induce progressively increasing reductions of the blood pressure. A steadily increasing and more protracted reduction in urine flow was observed when the individual was returned to the horizontal position.

In water loaded individuals the urine flow drops from 5 ml. per minute, or better, to 0.3 to 1 ml. per minute after syncope. This same urine flow is reached after syncope in subjects who have received no water load (Figure 2).

The specific gravity of urine during post-syncopal oliguria rises from low values to 1.020 to 1.025.

The inulin U/P ratio rises during oliguria to about 120, whereas in the control periods when the urine flow is at a rate of about 10 ml. per minute, the inulin U/P ratio has a value of about 12.

The subjective condition of the subject is greatly improved or essentially normal immediately after being returned to the horizontal position. It

FIG. 2. EXPERIMENT SHOWING THE INULIN AND DIODRAST CLEARANCE IN THE PASSIVE ERECT POSTURE AND AFTER A BRIEF COLLAPSE

Crosses: Plasma protein (per cent); open circles: Inulin clearance (ml. per min.); closed circles: Diodrast clearance (ml. per min.).

should be noted, however, that for the first 10 or 20 minutes after collapse there may be some pallor, due no doubt to capillary constriction in the skin.

Pulse rate and blood pressure return rapidly to normal after the subject is restored to the horizontal position.

The hemoconcentration which occurs in the passive erect posture (Figure 1) is replaced by a gradual dilution of the blood when the subject is returned to the horizontal position, the control values being reached in about 40 minutes.

Figure 2 shows the inulin clearance or glomerular filtration rate during a syncopal experiment. In this subject the bladder was catheterized and emptied with lavage. Immediately following syncope the filtration rate was reduced to about 70 per cent of its control value, whereafter it returned to the normal.

The diodrast clearance, or effective renal plasma flow, is also shown in Figure 2. The renal plasma flow was also reduced to about 75 per cent of its control value after syncope, returning later to its initial value. Our observations on the renal plasma flow and glomerular filtration rate are in agreement with other recorded data (2, 3).

Thus during the period of post-syncopal oliguria the subject feels quite well and the blood pressure, pulse, filtration rate and renal plasma flow are normal, while the hemoconcentration which appeared during the first phase of the oliguria is corrected. Having regard to these facts and to the high U/P ratio of inulin and the high specific gravity of urine, we infer that the mechanism governing the oliguria consists of a greatly increased reabsorption of water in the renal tubules. It may be supposed that this increased reabsorption of water occurs in consequence of an increased secretion of the antidiuretic hormone from the posterior lobe of the pituitary gland. Indeed, post-syncopal oliguria lasting 90 minutes is comparable with the effects of 3 to 4 i.u. of Insipidin (A.B.).[2]

DEMONSTRATION OF THE SECRETION OF THE ANTIDIURETIC HORMONE DURING POST-SYNCOPAL OLIGURIA

We have endeavored by various means to demonstrate the secretion of antidiuretic hormone dur-

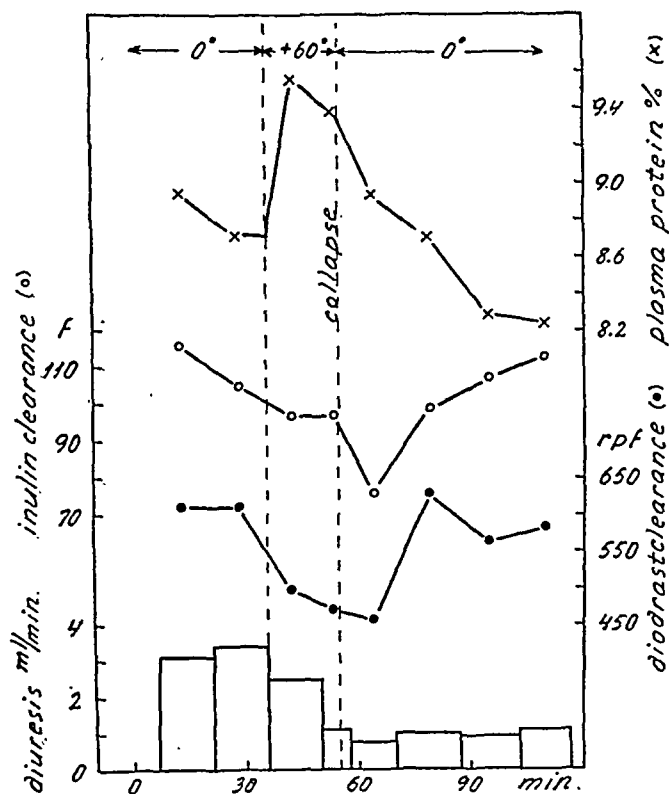[2] Insipidin A.B. (Alfred Benzon, Copenhagen). 1 ml. equals 20 i.u.

ing post-syncopal oliguria. First we tried to show that the effect on diuresis is of a humoral nature by transfusing blood from newly collapsed subjects. Next we tested the chloride output in the urine after collapse, since the antidiuretic hormone is supposed to influence the excretion of chloride by the kidney. Finally we induced collapse in 2 patients suffering from diabetes insipidus in order to find out if the post-syncopal oliguria is reduced in intensity or duration.

TRANSFUSION EXPERIMENTS

Blood transfusion from subjects with post-syncopal oliguria

The donors were normal subjects who had been loaded with 100 ml. of water every 10 minutes for a period; they were made to collapse on the tilt-table and in the course of 5 minutes after collapse, venesection was performed and 200 to 450 ml. of blood were withdrawn, coagulation being prevented by adding 15 mgm. of heparin to each 500 ml. of blood. The blood was transfused as quickly as possible (in the course of 5 to 10 minutes) to another subject who was in the horizontal position and in the state of constant diuresis. The blood pressure was followed during venesection and transfusion.

Figure 3 shows the diuresis curve of the recipient in 1 of these transfusion experiments. Prior to transfusion, the diuresis was fairly constant at about 14 ml. per minute. Two hundred ml. of blood were transfused from the subject who had just had a severe collapse, and in conjunction with which there was post-syncopal oliguria for 65 minutes. In the period immediately after the transfusion the recipient's urine flow fell to 2.9 ml. per minute, remaining at a reduced rate for about 45 minutes. During and after transfusion
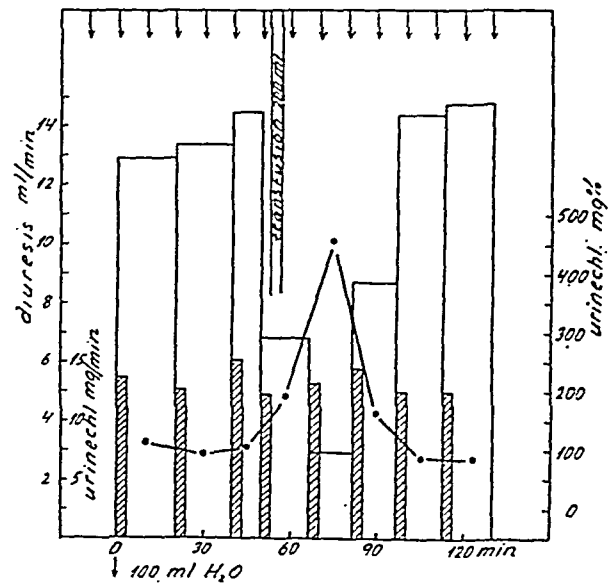

FIG. 3. DIURESIS AFTER TRANSFUSION OF 200 ML. BLOOD FROM A SUBJECT WITH POST-SYNCOPAL OLIGURIA

Closed circles: Urine chloride concentration (mgm. per cent); hatched columns: Chloride output in urine (mgm. per min.); plain columns: Urine flow (ml. per min.).

the recipient's blood pressure was unaffected. Table I gives the results of 3 experiments of this kind.

Control transfusion experiments

A total of 5 transfusion experiments were made using 2 recipients who were water loaded in the horizontal position in the usual manner. When the diuresis had become constant, venesection and transfusion of heparinized blood (275 to 450 ml.) were performed.

It will be seen from Table II that in none of the 5 experiments did the donors show a decreased urine flow after venesection. In 4 out of 5 experiments there was no significant decrease in

TABLE I

Experiments with transfusion of blood

	Donor			Recipient		
Blood pressure fall	Duration of oliguria (<2 ml. per min.)	Symptoms		Transfused volume of blood	Duration of oliguria	Fall of diuresis
	min.			ml.	min.	ml. per min.
132/92 to 80/62	90	Pallor, dizziness, deep inspirations, dimness of vision		400	30	11.3 to 4.5
112/75 to (0)?	38	Dimness of vision, oppression, no pulse		450	37	13.2 to 4.3
122/75 to ?	65	Complete collapse		200	47	14.5 to 2.9

TABLE II

Control experiments

Recipient				Donor		
Diuresis			Transfused volume of blood	Diuresis		
Before	During transfusion	After		Before	During venesection	After
ml. per min.	ml. per min.	ml. per min.	ml.	ml. per min.	ml. per min.	ml. per min.
12.2	10.8	11.5	450	13.1	11.3	10.7
16.3	17.0	17.5	300	15.6	13.6	16.0
10.4	8.4	7.7 to 10.2	300	14.5	15.0	16.7
16.5 to 19.5	20.5	19.5	175	13.6	6.3	16.8
14.3	5.8	7.3 to 12.1	300	14.4	17.3	13.0

urine flow in the recipients. In 1 experiment there was a moderate decrease in urine flow, from 14.3 to 5.8 ml. per minute, but it should be noted that because of poor veins the transfusion technique was not very successful, 3 painful punctures having to be made. The other experiments were unexceptional, technically.

These experiments show that a considerable reduction in urine flow can be brought about by transfusing blood from subjects who have just suffered syncope, whereas the transfusion of blood from control subjects had no such effect. The decrease in urine flow in the recipients is less and much shorter than the post-syncopal oliguria produced in the donors, but this is to be expected since only $\frac{1}{10}$ to $\frac{1}{20}$ of the blood volume is transfused.

We believe that the above evidence shows that the oliguria observed after circulatory collapse is caused by an antidiuretic substance in the blood, a substance that can be transferred by blood transfusion.

Urine chloride after administration of the antidiuretic hormone

Starling and Verney (4) experimenting with heart-lung-kidney preparations, showed that the addition of the posterior pituitary hormone to the blood brought about a greatly increased concentration of chloride, and that the absolute excretion of chloride per minute in the urine was increased (see also Shannon, 1942). Smith and MacKay (5) observed the same effects on man.

We have examined the plasma chloride concentration and the chloride concentration and the chloride ouput in the urine in a normal subject

after the intramuscular injection of 5 i.u. of Insipidin (A.B.) with the usual water load (100 ml. every 10 minutes). The chloride concentration in the urine during the ensuing oliguria rose considerably, whereas the per minute output fell. The urine chloride concentration remained considerably (about 40 per cent) above the plasma concentration as long as the oliguria lasted. The fall in chloride output observed by us is apparently contradictory to Smith and MacKay's observations, but one explanation may be that our subjects were in a state of pronounced negative chloride balance.

Urine and plasma chloride in post-syncopal oliguria

Figure 4 shows that the chloride concentration of the urine increases considerably during post-syncopal oliguria, reaching the value of 570 mgm. per cent, much higher than the plasma concentration. On the other hand, the chloride output does not rise: in fact, it has rather a tendency to fall. Two similar experiments gave this same result.

Urine and plasma chloride in oliguria transmitted by transfusion

Figure 3 shows the urine chloride concentration and the chloride output of a recipient during the
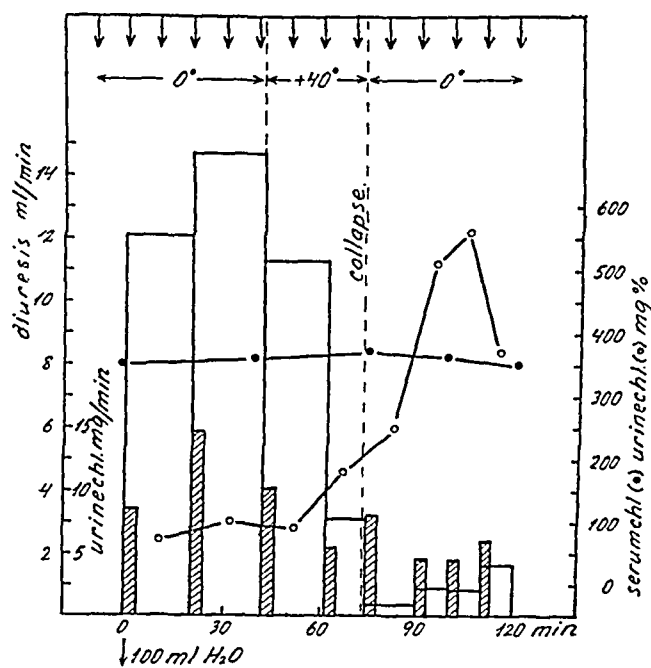

FIG. 4. DIURESIS AFTER CIRCULATORY COLLAPSE

Open circles: Urine chloride concentration (mgm. per cent); closed circles: Plasma chloride concentration (mgm. per cent); hatched columns: Chloride output in urine (mgm. per min.).

oliguria transmitted by blood transfusion. Here we find the same phenomenon, namely, a marked increase in urine chloride concentration, this value exceeding the blood chloride concentration. The chloride output, however, remains almost constant.

Although in our experiments the chloride output has not increased during post-syncopal oliguria, we consider that our results constitute circumstantial evidence in support of our hypothesis. We attach importance to the fact that the chloride output after the infusion of Insipidin and during post-syncopal oliguria behaves in the same manner under our experimental conditions. The fact that we found no increased chloride output in either circumstance may, as already stated, be due to the fact that our subjects were in a state of negative chloride balance owing to the heavy water load.

Circulatory collapse in patients with diabetes insipidus

Two young, otherwise healthy men with diabetes insipidus were employed as experimental subjects. Both subjects reacted positively to posterior pituitary extract, and their 24-hour urine output when not receiving extract was 6 to 14 liters respectively.

Both subjects reacted in the same manner after syncope. They showed a decreased urine flow of the same order of magnitude as shown by normal persons, and the inulin U/P ratio rose just as high (to 120). On the other hand, the duration of oliguria in both subjects was about 20 minutes, a much shorter period than is observed in corresponding experiments on normal subjects (60 to 90 minutes). The blood pressure fell as in normal subjects; likewise, the clinical condition during collapse was comparable with that of the completely collapsed normal subjects, and they recovered just as quickly as the normals after return to the horizontal.

It should be noted that diabetes insipidus was slight in one of our subjects and only moderately severe in the other, and hence we would not expect to find any excessive deviation in behavior from the normal. Nevertheless, we believe that the shorter duration of the oliguria in the subjects with diabetes insipidus must be attributed to their inability to produce antidiuretic hormone in the same quantities as normals do in the same situation.

Summarizing the results of the 3 series of experiments, none of which taken separately can be said to constitute absolute proof of the correctness of the theory, but all of which point in the same direction, we believe that we have produced evidence that post-syncopal oliguria is caused by an increased secretion of the antidiuretic hormone. Nothing can be concluded from our experiments as to the nature of the releasing mechanism operating on pituitary secretion. The effective stimulus might be either cerebral anoxia, caused by the fall of blood pressure, or a reflex effect on the pituitary gland mediated through the pressor receptors.

Finally, some brief reference may be made to what we imagine may be the effect on the organism of this pituitary regulation of diuresis as demonstrated in the case of circulatory collapse.

In the passive erect posture the circulating blood volume is reduced sometimes to catastrophically low values, the result being syncope. Regulation of the diuresis may possibly contribute towards reestablishing the normal blood diuresis, but quite quantitatively it can scarcely be of much importance when the diuresis is of the usual volume.

The regulation may perhaps be regarded merely as an *accompanying phenomenon* to another pituitary regulation, first and foremost a regulation of the tone of the capillaries, which would be appropriate in a situation such as that described. It should be mentioned in this connection that the subjects were always rather pale for some time after the syncope, a sign of capillary contraction in the skin.

It may be presumed that the hormonal regulation of diuresis may be contributory to the oliguria observed in cases where there is circulatory insufficiency, for example, hemorrhage, fall of blood pressure and shock as in lumbar anesthesia, shock caused by burns, cardiac insufficiency, diabetic coma, etc.

SUMMARY

Brief circulatory collapse brought about in water-loaded subjects by means of a tilt-table is followed by a protracted period of oliguria (post-syncopal oliguria). The degree and duration of

the oliguria seem to depend upon the degree of circulatory collapse.

Since the rate of glomerular filtration is rapidly restored to normal after the syncopal period, the oliguria may be attributed to an increased reabsorption of water by the renal tubules.

It is suggested that post-syncopal oliguria is due to an increased secretion of the antidiuretic, posterior pituitary hormone. This hypothesis is supported by the following experiments:

1. The transfusion of blood from subjects who had just collapsed with post-syncopal oliguria into water-loaded subjects caused a distinct decrease in urine flow in the latter.

2. The increased chloride concentration of the urine and the rate of chloride excretion during post-syncopal oliguria corresponds to the changes observed after administration of posterior pituitary extract.

3. Circulatory collapse in 2 patients with moderate diabetes insipidus was followed by post-syncopal oliguria, which was of considerably shorter duration than in normal persons.

The initiating mechanism of post-syncopal oliguria and its possible clinical relations to other forms of oliguria are discussed.

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THE FATE OF INTRAVENOUSLY INJECTED GELATIN IN HUMAN SUBJECTS [1]

By WILLIAM S. HOFFMAN AND DONALD D. KOZOLL [2]

(*From the Hektoen Institute for Medical Research of the Cook County Hospital and from the Departments of Surgery of the Cook County Hospital and the Northwestern University Medical School, Chicago*)

The value of specially prepared gelatin solutions for intravenous infusion as a plasma substitute in the treatment of shock caused by trauma or hemorrhage is now well recognized. Previous papers from our laboratory (1 to 3) have reviewed the literature on the subject and have presented findings indicating the effectiveness of such solutions in various types of shock encountered in human subjects in a large general hospital. These reports have demonstrated that intravenously injected gelatin is an effective and innocuous hemodiluting agent not only in patients with shock but in the relatively normal "hospital control" subjects.

The present investigation was undertaken to determine the fate of intravenously injected gelatin; the plasma levels achievable; the distribution of gelatin in the blood, tissues, and urine; and the rate of gelatin excretion. An effort was made also to determine to what extent the rate of excretion of gelatin varied with preparations of varying molecular weight.

PLAN OF STUDY

The gelatin preparations [3] used in this study were all 5 per cent solutions of osseous gelatin prepared by electrodialysis of calcium gelatinate. They were of three stages of degradation. Lots No. 39, 45, 65, and 80 were highly degraded gelatins prepared by twice autoclaving for 30 minutes at 15 pounds pressure. These had broad ultracentrifuge patterns and relatively low viscosity. Their weight average molecular weight was of the order of 37,000. It was this type which had been used exclusively in the previous studies from this laboratory and which had been found clinically effective. Lots No. 58–10, 83, and 93 were much less degraded. They were prepared by autoclaving for 20 minutes at a pressure of 10 pounds. Their weight average molecular weight was of the order of 58,000. Lots No. 58–15 and 101 were intermediate with a weight average molecular weight of the order of 47,000. They were autoclaved for 20 minutes at 15 pounds pressure. All of these solutions, it should be recognized, including the least degraded, remained liquid at room temperatures. The latter were slightly more degraded than the Knox preparation employed by Koop (5) and others, which is gelled at room temperatures and must be warmed to body temperature before utilization.

To avoid cumbersome repetition, the least degraded preparations used in this study will be spoken of as heavy gelatin; the most highly degraded, as light gelatin; and the intermediately degraded, as intermediate gelatin.

All preparations except one were in physiological saline solution; Lot No. 39 was 5 per cent gelatin in 5 per cent dextrose solution. These solutions were stable at room temperatures showing no discoloration or other change even after a year. The older as well as the fresh preparations, as in previous studies, produced no reactions, except in the case of one lot (Lot. No. 80), which produced mild pyrogenic reactions that could not be attributed to faulty tubing or glassware.

The subjects chosen for this study were so called hospital controls, patients in the convalescent stage of illnesses not likely to produce appreciable changes in cardiac, circulatory, or renal function. They were for the most part patients recuperating from herniorrhaphy. All were

of Scatchard *et al.* (4). These values are listed in the following table:

Lot. No.	H_o	M_w
B20610–39	0.2202	37,266
B20610–45	0.2100	35,532
B20610–65	0.2176	36,820
B20610–80	0.2248	38,040
B20610–58–15	0.2853	48,270
B20610–101	0.2683	45,450
B20610–58–10	0.3345	56,600
B20610–83	0.3391	57,600
B20610–93	0.3592	59,254

For the sake of convenience, the prefix B20610– will be omitted in references to the individual lots.

[2] Abbott Fellow in Surgery, Northwestern University Medical School.

[3] The gelatin solutions were kindly furnished by the Upjohn Company. They came in liter bottles prepared for direct use in intravenous infusions. They contained no preservative. The weight average molecular weight (M_w) of the various lots were determined by the Upjohn Company from the intrinsic viscosity (H_o) by the method

males. In a preliminary study a group of 24 subjects were given 2000 ml. of 5 per cent gelatin and the blood and urine gelatin concentration determined after the injection of 1000 ml. and after 2000 ml., and at 24, 48, 72, and 96 hours. Later, it was recognized that a much better insight into the fate of the injected gelatin could be obtained by the following procedure: At 9 a.m. (some 2 hours after breakfast), a control sample of blood was obtained and the patient was asked to empty his bladder. Then 1000 ml. of 5 per cent gelatin were injected at the usual clinical speed of about 330 ml. per hour. At the end of the injection, a blood sample was taken and the accumulated urine collected without catheterization. Then blood and total urine samples were collected at 2 hours, 4 hours, and 6 hours after the end of the injection, and again at 24, 48, 72 and at times 96 and 120 hours after the start of the injection. Gelatin and creatinine determinations were made on all blood and urine samples, thus a comparison of creatinine and gelatin clearances was possible. Besides, the 24-hour creatinine excretions served as a check on the completeness of the urine collections. Those experiments in which there was some doubt as to the accuracy of the collection were discarded. Since the data in the 24-hour preliminary experiments in which 2000 ml. of gelatin were injected do not lend themselves to the same type of analysis as the later experiments, they will not be presented. It should be mentioned, however, that the injection of 2000 ml. was as innocuous as that of 1000 ml. and that the general findings in these experiments, both as to blood levels and rates of excretion, were consonant with the 42 more complete later experiments.

Gelatin was determined in blood and urine by the method of Janota (6) as follows: the total nitrogen (TN) was first determined by micro Kjeldahl method with distillation and microtitration. From this value was subtracted the gelatin plus non-protein (GN) obtained by Kjeldahl determination of a trichloroacetic acid filtrate. The difference is the albumin and globulin nitrogen. The non-protein nitrogen (NPN) was determined in an alcohol filtrate. The albumin and non-protein nitrogen (AN) was obtained by analysis of a filtrate obtained by addition of 21 per cent sodium sulfite (7). Thus

$$TN - GN = \text{Serum Protein N}$$
$$AN - NPN = \text{Albumin N}$$
$$GN - NPN = \text{Gelatin N}$$
$$\text{Serum Protein N} - \text{Albumin N} = \text{Globulin N.}$$

Albumin, globulin, and serum protein N were multiplied by 6.25 to give values as protein, while gelatin N values were multiplied by 5.7 to give gelatin values. As a check on the total nitrogen determinations in the serum, total protein determinations were also made by the falling drop method of Barbour and Hamilton (8). These usually agreed well with the sum of the serum protein plus gelatin concentrations, indicating that for the relatively small concentrations of gelatin, very little error was introduced by calculating the gelatin influence on the specific gravity as equal to that of the same weight of serum protein. Since it was found that the urine of these sub-

jects never contained any protein precipitable by trichloroacetic acid, urinary gelatin N could be determined by subtracting the NPN from the total N without any appreciable error.

Creatinine determinations were made in plasma and urine by a photoelectric modification (9) of the Folin method. Plasma volume estimations were carried out by the photoelectric Evans Blue method in which a control and single 10-minute sample were taken, as suggested by Gregersen (10). The determinations were made just before the injection of gelatin and immediately after the end of the injection.

RESULTS

Plasma gelatin concentration. The data for 20 cases of heavy gelatin injections, 9 intermediate, and 13 light gelatin injections are given in Tables IA, IB, and IC respectively. The highest plasma gelatin concentration, achieved at the end of the injection, ranged between 0.59 to 1.17 grams per 100 ml. The averages for the three types were almost identical (0.78, 0.79, and 0.78 gram per 100 ml. respectively). There was some indication that the levels were proportional to the speed of the injection; but this impression was not con-

TABLE IA

Plasma gelatin concentration after intravenous injection of 1000 ml. of 5 per cent "heavy" gelatin

Lot no.	Patient	Hours after completion of injection				
		0	2	4	6	24
		grams per 100 ml.	grams per 100 ml.	grams per 100 ml.	grams per 100 ml.	grams per 100 ml.
58–10	A.A.	0.82	0.74	0.57		0.29
	J.S.	0.83				0.32
	C.T.	0.72	0.60	0.58	0.51	0.33
	H.M.	0.91	0.75	0.72	0.52	0.43
	G.B.	0.86				0.41
	J.O.	0.77				0.44
	H.H.	0.63	0.50	0.49	0.47	0.24
	O.B.	0.75	0.61	0.48	0.46	0.32
	F.B.	0.76	0.61	0.60	0.53	0.31
	J.D.	0.74	0.64	0.59	0.54	0.35
	A.W.	0.74	0.68	0.62	0.53	0.40
	L.Y.	0.88				0.38
	A.D.	0.75				0.27
	A.E.	0.69				0.38
83	W.H.	0.86	0.82	0.66	0.47	0.43
93	R.R.	0.77	0.74	0.69	0.62	0.40
	D.C.	0.93	0.72	0.71	0.58	0.29
	C.W.	0.79	0.64	0.65	0.58	0.41
	P.W.	0.68		0.55	0.55	0.35
	J.R.	0.74	0.72	0.71		0.29
Average and standard deviation		0.78 ±0.07	0.66 ±0.08	0.64 ±0.08	0.52 ±0.05	0.35 ±0.06

TABLE IB

Plasma gelatin concentration after intravenous injection of 100 ml. of 5 per cent "intermediate" gelatin

Lot no.	Patient	Hours after completion of injection				
		0	2	4	6	24
		grams per 100 ml.	grams per 100 ml.	grams per 100 ml.	grams per 100 ml.	grams per 100 ml.
58–15	O.B.	0.82	0.67	0.61	0.58	0.11
	H.C.	0.75	0.59	0.59	0.53	0.16
	S.S.	0.88	0.67	0.65	0.57	0.35
	F.F.	0.92	0.74	0.65	0.62	0.44
101	J.M.	0.95	0.64	0.59	0.55	
	R.Y.	0.73	0.49	0.43	0.33	
	H.M.	0.64	0.38	0.35	0.31	
	F.B.	0.68	0.43	0.33	0.29	0.12
	W.C.	0.74	0.55	0.46	0.41	0.13
Average and standard deviation		0.79 ±0.10	0.57 ±0.11	0.52 ±0.12	0.47 ±0.12	0.22 ±0.13

TABLE IC

Plasma gelatin concentration after intravenous injection of 1000 ml. of 5 per cent "light" gelatin

Lot no.	Patient	Hours after completion of injection				
		0	2	4	6	24
		grams per 100 ml.	grams per 100 ml.	grams per 100 ml.	grams per 100 ml.	grams per 100 ml.
39	J.C.	0.59	0.51	0.36	0.36	0.34
	C.U.	0.69	0.51	0.41	0.32	0.20
	L.B.	0.78	0.63	0.45	0.45	0.42
45	S.M.	0.90	0.62	0.56	0.53	0.37
	C.P.	0.84	0.60	0.51	0.47	0.35
65	L.P.	1.17	0.67	0.61	0.51	0.40
	L.M.	0.77	0.55	0.51	0.46	0.33
	Lu.M.	0.68	0.43	0.42	0.27	0.14
	J.W.	0.78				0.48
80	A.T.	0.67	0.52	0.45	0.42	0.34
	H.R.	0.87	0.55	0.52	0.46	0.40
	J.S.	0.88	0.52	0.43	0.27	0.24
	B.C.	0.71	0.54	0.52	0.39	0.08
Average and standard deviation		0.79 ±0.14	0.55 ±0.06	0.48 ±0.07	0.41 ±0.095	0.32 ±0.11
t (heavy-light)		0.3	4.5	6.0	4.3	1.0

firmed, for no attempt was made to vary the speed of injection.

In a few cases plasma gelatin levels were determined during the administration of the gelatin. These values were always lower than those obtained at the end of the injection. Similarly, in the preliminary series of experiments in which

2000 ml. were injected, the plasma gelatin concentration reached at the end of 1000 ml. was always lower than that obtained at the end of 2000 ml. Thus it was certain that the rate of removal of gelatin from the blood stream was always slower than that of the ingress, if the injection was made at the common clinical speed.

The plasma gelatin concentrations dropped progressively in 24 hours to 0.35, 0.22, and 0.32 mgm. per 100 ml. respectively for the heavy, intermediate, and light gelatins. There were still appreciable concentrations of gelatin in the plasma at 48 and 72 hours, which finding was confirmed by the demonstrable excretion of gelatin on the third and fourth days. Though a comparison of the levels at the end of the injection and at 24 hours did not reveal any appreciable differences between the heavy and light gelatin (the *t* value of statistically significant difference [4] being 0.3 for 0 hour and 1.0 for 24 hours), such a comparison made at 2, 4, and 6 hours showed that the rate of fall in plasma gelatin concentration during this period was far greater for the light gelatin than for the heavy variety. This difference is apparent in the curves of plasma gelatin concentration shown in Figure 1. That the difference is not fortuitous is indicated by the *t* values of 4.5 at 2 hours, 6.0 at 4 hours, and 4.3 at 6 hours. The heavy gelatin thus tended to maintain an effective concentration longer than did the light gelatin. A possible explanation for this phenomenon will become recognizable when the excretion data are analyzed. The plasma gelatin concentrations at 2, 4, and 6 hours for the intermediate types appeared to fall in between those of the other two types, but the differences were less significant chiefly because

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[4] The *t* value of statistically significant difference between the average of two sets of experimental values was determined according to the method of Snedecor (11) as follows:

$$t = \frac{c_1 - c_2}{\sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}}$$

where c_1, σ_1, and n_1 are respectively the mean, the standard deviation, and the number of experiments in the one set of experiments, and c_2, σ_2, and n_2 are those in the second group of experiments. A value of 2.5 or more for *t* indicates that the difference of the means is at least 4 times as great as the probable error of the difference of the means and is therefore probably a significant difference.
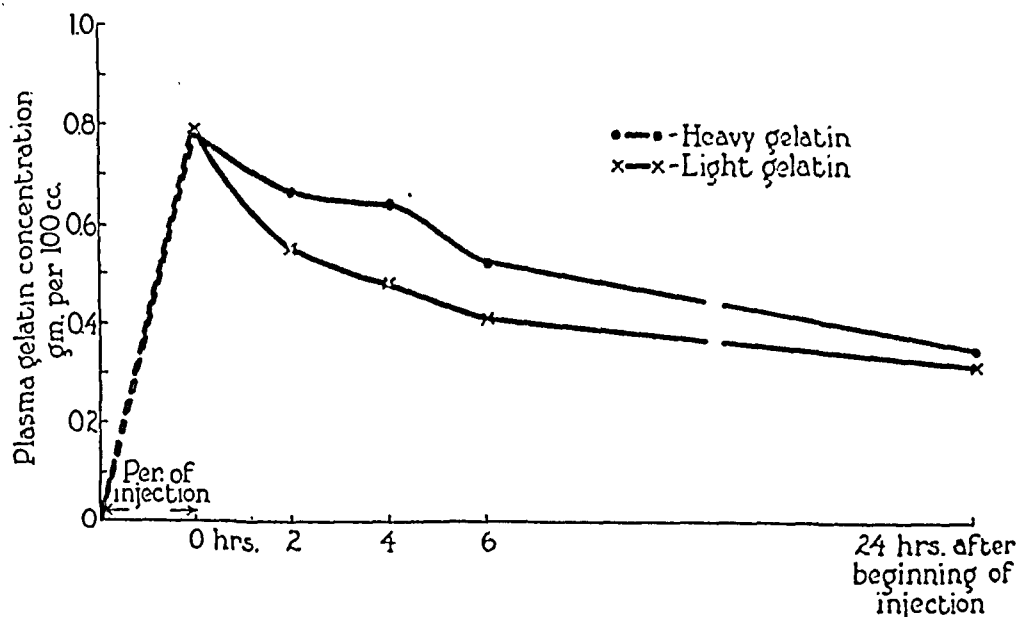
FIG. 1. COMPARISON BETWEEN AVERAGE PLASMA GELATIN CONCENTRATIONS AFTER
INJECTION OF HEAVY GELATIN AND THOSE AFTER LIGHT GELATIN

the variations were greater for the intermediate type.

Urinary excretion of gelatin. The data for the excretion of gelatin in the urine for 14 heavy gelatin injections, 9 intermediate and 12 light gelatin injections are shown in Table IIA, IIB, and IIC respectively. It is obvious that the rate of excretion shows a marked variability even in injections of the same lot of gelatin. However a pattern of excretion is discernible. In all cases there was a considerable excretion of gelatin by the time of the end of the injection. At 6 hours after the com-

pletion of the injection an average of 22.72 grams of light gelatin had been excreted. The heavy gelatin excretion in this period was significantly less, averaging 14.68 grams. The intermediate gelatin excretion was 21.72 grams. There was much less variation in the individual excretion of the heavy gelatin at 6 hours than in those of light gelatin, the standard deviation being 2.50 while that for the light gelatin was 4.22. These findings were consistent with the differences in plasma levels by the end of 6 hours. That the difference between the excretion of heavy and light gelatin

TABLE IIA

Urinary excretion of "heavy" gelatin after intravenous injection of 1000 ml. of 5 per cent solution

	Lot no. 58–10								Lot no. 93				Lot no. 83		Average	Standard deviation
	C.T.	H.M.	A.H.	F.B.	A.A.	J.D.	A.W.	O.B.	D.C.	R.R.	C.W.	P.W.	J.R.	W.H.		
	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams
At end of injection	6.36	7.85	1.38	1.85	2.41	7.15	6.47	7.29	6.20	14.63	17.45	8.87	6.89	5.56		
2 hrs. after	4.39	3.78	2.87	4.75	7.92	1.23	5.21	3.76	5.16	5.52	4.13		2.27	3.19		
4 hrs. after	1.81	4.63	4.91	2.98	4.63	1.60	2.90	1.72	2.54	1.81	2.20	0.92	2.52	3.34		
6 hrs. after	3.74	2.69	1.36	3.17	2.41	4.69	1.25	1.07	0.98		1.15	0.88		3.53		
Total to 6 hrs.	16.30	18.95	10.52	12.75	17.37	14.67	15.83	13.84	14.98			10.67		15.62	14.68	±2.50
24 hrs.		7.81	15.55	7.93	8.36		8.89	16.51	5.23	5.92	1.78	14.44	23.38	6.37		
48 hrs.		9.44	7.76	18.15	4.53		16.52	6.64	3.37	5.83			4.00	14.35		
72 hrs.		6.03		2.96	4.33		3.21	5.30	8.62	1.29			0.39			
Total to 72 hours		42.23		41.79	34.59		44.45	42.30	32.20	34.90			39.47		39.00	±4.21

t (Average "heavy" gelatin excretion to 6 hours compared with average "light" gelatin excretion to 6 hours) 5.6.
t (Average "heavy" gelatin excretion to 72 hours compared with average "light" gelatin excretion to 72 hours) 0.7.

TABLE IIB

Urinary excretion of "intermediate" gelatin after intravenous injection of 1000 ml. of 5 per cent solution

	Lot no. 58–15				Lot no. 101					Average	Standard deviation
	U.B.	H.C.	S.S.	F.F.	J.M.	R.Y.	M.H.	F.B.	W.C.		
	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams
At end of injection	22.06	10.18	7.17	4.63	14.96	7.99	10.29	5.60	8.18		
2 hrs. after	2.31	5.56	4.73	5.75	8.13	9.51		8.81	10.56		
4 hrs. after	2.08	2.41	4.77	7.33		2.56	5.69	2.45	3.41		
6 hrs. after	1.63	1.31	1.17	2.95	5.92	1.61	1.85	1.33	0.58		
Total to 6 hrs.	28.08	19.46	17.84	20.66	29.01	21.67	17.83	18.19	22.73	21.72	±4.00
24 hrs.	2.82	3.24	4.06	3.75	6.72	6.16	7.98	8.09	5.70		
48 hrs.	5.67	1.88	10.94	8.29							
72 hrs.	5.80	7.55	4.91	2.79							
Total to 72 hrs.	42.37	32.13	37.75	35.49						36.69	
96 hrs.	1.15	3.94	8.19	0.54							

TABLE IIC

Urinary excretion of "light" gelatin after intravenous injection of 1000 ml. of 5 per cent solution

	Lot no. 39			Lot no. 65			Lot no. 45			Lot no. 80			Average	Standard deviation
	J.C.	C.U.	L.B.	L.P.	L.M.	LU.M.	S.M.	C.P.	A.F.	H.R.	J.S.	A.D.		
	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams
At end of injection	18.24	12.97	12.19	7.70	5.65	12.14	14.50	8.84	9.70	12.41	9.23	9.25		
2 hrs. after	7.01	8.70	10.06	9.32	3.04	7.34	6.53	8.90	4.22	3.02	4.65	5.58		
4 hrs. after	2.53	3.41	2.80	4.01	2.14	4.39	3.59	2.66	1.11	2.48	3.10	1.71		
6 hrs. after	3.58	1.51	1.43	1.90	6.41	1.20	1.62	1.01	3.46	0.73	1.59	3.10		
Total to 6 hrs.	31.36	26.59	26.48	22.93	17.24	25.07	26.21	21.41	18.49	18.64	18.57	19.64	22.72*	±4.22
24 hrs.	8.72	4.59	5.79	9.10	6.30	2.33	6.74	6.96	7.02	15.20	23.37	17.60		
48 hrs.	1.73	3.24	2.17		4.61	4.02	3.23	5.74	2.07	2.58	1.54	2.79		
72 hrs.	0.73	2.41			15.81		1.63		8.86	1.04	0.08	2.10		
Total to 72 hrs.	42.54	36.83			43.96		38.84		36.44	37.46	43.56	42.13	40.22	±2.93

* If lot 80 is omitted, average excretion to 6 hours is 24.66.

was significant was shown by the *t* value of 5.6. By the end of 72 hours, the differences in excretion had largely disappeared. After the 6-hour period, the heavy gelatin began to be excreted more rapidly than the lighter gelatin, so that at 72 hours, an average of 39.00 grams was accounted for in the urine, while for the light gelatin 40.22 grams were excreted. The *t* value for these differences was only 0.7 which means that the difference was probably statistically insignificant.

For the 9 cases of injection of intermediate gelatin, there were not enough data at 72 hours to allow statistical evaluation, but here, too, it appeared that the final total excretion was of the same order as that for the heavy and light gelatin.

In a few cases determinations of gelatin excre-

tion were made for the fourth day. An appreciable quantity of gelatin was invariably recovered, occasionally as much as 5 grams. Traces of gelatin were found in the urine on the fifth day, but the values often lay within the probable error of the determination. Since some 80 per cent of the injected gelatin could be accounted for in the urine in 72 hours, and since gelatin was still being excreted on the fourth, and presumably on the fifth day, it was probable that little or no gelatin was degraded to products smaller than those precipitated by alcohol. The lack of any significant rise in serum NPN and urea N during or after injection was in agreement with this interpretation, which is contrary to the conclusion arrived at by Brunschwig (12).